Aus der Klinik für Allgemein-/ Viszeral- und Transplantationschirurgie

Klinik der Universität München

Vorstand: Prof. Dr. Jens Werner

New Prognostic and Predictive Biomarkers in Primary Ovarian Cancer

Dissertation
zum Erwerb des Doktorgrades der Medizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von

Katharina Anna Dötzer

aus

Forchheim

Jahr

2022

Mit Genehmigung der Medizinischen Fakultät
der Universität München

Berichterstatter:	PD Dr. rer. nat. Barbara Mayer
Mitberichterstatter:	Prof. Dr. med. Fabian Trillsch
	PD Dr. med. Julia Jückstock
	Prof. Dr. Robert Pernenczky
Mitbetreuung durch den promovierten Mitarbeiter:	Prof. Dr. med. Nina Ditsch
Dekan:	Prof. Dr. med. Thomas Gudermann
Tag der mündlichen Prüfung:	08.12.2022

Affidavit

Eidesstattliche Versicherung

Dötzer, Katharina Anna

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Titel:

New Prognostic and Predictive Biomarkers
in Primary Ovarian Cancer

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, 10.01.2023
Ort, Datum

Katharina Dötzer
Unterschrift Doktorandin

Table of Content

List of Abbreviations

BRCA: Breast cancer gene

CAM-DR: Cell-adhesion mediated drug resistance

CD: Cluster of differentiation

DCR: Disease Control Rate

ECM: Extracellular matrix

EGFR: Epidermal growth factor receptor

ERα: Estrogen receptor alpha

FIGO: Fédération Internationale de Gynécologie et d'Obstétrique

HGFR: Hepatocyte growth factor receptor

NSCLC: non-small-cell lung cancer

ORR: Overall Response Rate

OS: Overall Survival

PD-1: Programmed cell-death protein 1

PD-L1: Programmed cell-death ligand 1

PFI: Platinum Free Interval

PFS: Progression Free Survival

QTiS: Quantification of the tumor immune stroma

SCLC: small-cell lung cancer

TAM: Tumor associated macrophage

TIL: Tumor infiltrating Lymphocyte

VEGF: Vascular Endothelial Growth Factor

WHO: World Health Organization

List of Publications

Paper 1:

Dötzer, K.; Schlüter, F.; Schoenberg, M.B.; Bazhin, A.V.; Koch, F.E.v.; Schnelzer, A.; Anthuber, S.; Grab, D.; Czogalla, B.; Burges, A.; Werner, J.; Mahner, S.; Mayer, B.

Immune Heterogeneity Between Primary Tumors and Corresponding Metastatic Lesions and Response to Platinum Therapy in Primary Ovarian Cancer.

Cancers (Basel) 2019, 11, 1250, doi:10.3390/cancers11091250.

Paper 2:

Dötzer, K.; Schlüter, F.; Koch, F.E.v.; Brambs, C.E.; Anthuber, S.; Frangini, S.; Czogalla, B.; Burges, A.; Werner, J.; Mahner, S.; Mayer, B.

Integrin α2β1 Represents a Prognostic and Predictive Biomarker in Primary Ovarian Cancer.

Biomedicines 2021, 9, 289, doi:10.3390/biomedicines9030289.

1. Introduction

1.1. Ovarian Cancer

1.1.1.Epidemiology

In 2020, worldwide ovarian cancer incidence was 313,959 and 207,252 deaths as a result were counted. In Germany there have been 7,162 new cases and 5,328 deaths [1]. This makes ovarian cancer to the second leading death cause among gynecologic cancers. As the incidence rates increase with age, the average age at time of diagnosis is 68 years. One in 76 women will suffer from ovarian carcinoma during their lifetime, which results in a lifetime prevalence of 1.3% [2].

1.1.2.Clinical Manifestation and Classification

Currently, there are no established screening methods that can detect ovarian cancer in early stages [3,4]. Usually, unspecific symptoms occur in advanced stages, leading to late diagnosis for the majority of patients [5]. Around 75% of the patients receive diagnosis in stage III and IV (see Table 1), in which tumor mass has usually spread in the whole peritoneal cavity.

FIGO-Stage	Definition	Distribution at initial diagnosis
I	Tumor confined to ovaries or fallopian tubes	22%
II	Tumor with pelvic extension (below pelvic brim)	7%
III	Tumor with spread outside the pelvis	43%
IV	Distant metastasis (excluding peritoneal metastases)	29%

Table 1: FIGO staging classification for ovarian cancer (according to [2,6])

1.1.3. Therapy (according to German Guidelines)

Primary surgery combines staging and radical tumor debulking in advanced stages. An open laparotomy allows exploration of the complete peritoneal cavity. Peritoneal cytology, multiple peritoneal biopsies, bilateral salpingo-oophorectomy, total abdominal hysterectomy, appendectomy, omentectomy and pelvic and paraaortal lymph node dissection are conducted to define tumor stage. Further, macroscopic tumor should be completely removed by radical debulking [4]. Despite rigorous surgical resection around 50 to 60% of patients suffer from macroscopic residual tumor burden, which is associated with poor prognosis [7-9].

After primary surgery in nearly all cases (except stage IA/IB and grading G1/G2) an adjuvant first-line systemic chemotherapy is recommended. It should consist of six cycles carboplatin combined with paclitaxel. Due to high toxicity and severe side effects of this drug combination, chemotherapy has to be terminated early for more than 10% of patients, which is associated with poor prognosis. Further

dose modification is required even more frequently [8,10,11]. To improve prognosis, a large number of studies was conducted, evaluating the effect of targeted therapies in ovarian cancer. So far, only a small number of candidates could be included in the guideline recommendations [12-14].

In advanced stages, Bevacizumab, a VEGF-inhibitor, can be administered simultaneously to chemotherapy and as maintenance therapy for up to 15 months. For patients with BRCA-mutation the PARP-inhibitor Olaparib can be applied as single maintenance therapy or in combination with Bevacizumab. Recently, the PARP-inhibitor Niraparib is recommended for all patients in advanced stages independent of BRCA-mutation status [4]. Currently many phase-III-trials are ongoing, evaluating the efficacy of various PARP-inhibitors in combination with chemotherapy or other targeted therapies, like checkpoint-inhibitors (NCT03642132, NCT03602859, NCT03737643, NCT03522246).

In case of recurrence, further therapy options depend on the interval from completion of chemotherapy to relapse. There are four categories: Platinum-refractory (no response to chemotherapy, relapse < 4 weeks), platinum-resistant (relapse < 6 months), partial platinum-sensitive (relapse 6-12 months), platinum-sensitive (relapse > 12 months) [4]. Especially for platinum-refractory and platinum-resistant disease platinum-free monotherapy is recommended for second-line therapy. Recently, the definition of 'platinum-resistant' has begun to change. Instead of strictly using a cut-off of six months for selection of second-line therapy, more factors should be included e.g., age and performance status of the patient, patient's preference, histology, and BRCA-mutation. Nevertheless, there are no established markers, to predict response to platinum-based first- or second-line therapy [15]. Due to frequent relapse, predictive markers could help to stratify patients for effective therapies and thus improve poor prognosis.

1.1.4.Prognosis and Prognostic Factors

The 5-year-survival rate of ovarian cancer is stage-dependent. For patients in stage III at the time of diagnosis its 39%, in stage IV it is 20% [2]. Low survival rates originate from frequent and early relapses after chemotherapy. 70 to 80% of patients suffer from a relapse. In most patients it occurs within the first three years after chemotherapy [16,17].

There are several proven prognostic clinicopathological factors. High age and a low performance status of the patient, high tumor grading and advanced tumor stage are associated with poor prognosis [4,18,19]. The presence of macroscopic residual tumor after surgery is another important prognostic factor. Best prognosis can be expected after optimal cytoreduction. If the amount of macroscopic residual tumor is less than 10mm, prognosis is still better than with more residual tumor [7,20]. Consequently, prognosis also depends on the quality of primary surgery.

Additional to clinicopathological factors recently factors concerning tumorbiology have proven their prognostic value. The new WHO classification defined the histologic subtypes by molecular patterns, differing in survival rates. Certain gene signatures and cell cycle proteins were found to correlate with prognosis [21,22]. As it has been already proven for other cancer entities, high counts of TILs are associated with better prognosis [23,24].

In summary the poor prognosis of ovarian cancer and the lack of biomarkers for patient stratification indicates the high medical need for new prognostic and predictive factors. Identification of those was the purpose of the present work and is summarized in two publications. In the paper “Immune Heterogeneity Between Primary Tumors and Corresponding Metastatic Lesions and Response to Platinum Therapy in Primary Ovarian Cancer” the immune infiltrate in the microenvironment of primary ovarian cancer was analyzed. The protein expression pattern of drugable targets was investigated in the paper “Integrin α2β1 Represents a Prognostic and Predictive Biomarker in Primary Ovarian Cancer”.

1.2. Immune Infiltrate in Ovarian Cancer

1.2.1. Microenvironment in Ovarian Cancer

Many characteristics and features of ovarian cancer are connected to its tumor microenvironment. Single tumor cells and aggregates separate from the primary tumor, resulting in mainly peritoneal and omental metastases [25]. Components of the ovarian cancer microenvironment are (1) the ECM, (2) residential cells at the site of metastasis, like mesothelial cells or omental adipocytes, or (3) recruited cells, like fibroblasts, macrophages, and other immune cells. By interaction with ovarian cancer cells, they can be reprogrammed and therefore enable chemotaxis, adhesion, tumor growth, angiogenesis, metastasis, immune modulation and chemoresistance [26-28].

1.2.2. Tumor-Infiltrating Lymphocytes in Ovarian Cancer

As a part of the tumor microenvironment immune cells can enable, but also inhibit tumor growth. While TAMs or regulating T-cells induce immunosuppression, TILs contribute to antitumoral activity [29]. Meta-analyses have proven the prognostic value of TILs. A high amount of intraepithelial $CD3^+$ or $CD8^+$-cells is associated with better PFS and OS in ovarian cancer [23,24].

1.2.3. Relevance of Checkpoint-Inhibitors in Ovarian Cancer

PD-1 and its ligand PD-L1 play an important role in regulation of the immune system and tolerance of T-cells. Binding of the two ligands leads to suppression of immune response in the PD-1 expressing T-cell. Usual PD-L1-expression on antigen-presenting cells or tissue in sites of immune privilege, like placenta or testes, leads to immune regulation by the PD-1 pathway. But expression on tumor cells of various entities is frequent and therefore decreases antitumoral activity of the peritumoral immune infiltrate [30-32].

Monoclonal antibodies, targeting PD-1 and PD-L1, were developed to restore antitumoral activity of T-cells. In recent years, the development of checkpoint-inhibitors led to an improvement of cancer therapy in many entities. They are part of the first-line therapy for NSCLC, SCLC, renal cell carcinoma and malignant melanoma [33-35]. Due to the discovery of the role of TILs for antitumoral activity in ovarian cancer, checkpoint inhibitors have become promising therapeutic options.

Initial studies of checkpoint inhibitors in recurrent ovarian cancer showed ORRs of 10 to 15% with DCRs of around 40 to 50% [36-38]. KEYNOTE-100 was the first large, phase-II-trial. Pembrolizumab, a

PD-1 inhibitor, was administered to patients with recurrent ovarian cancer, showing an ORR of 8% and DCR of 37%. In this study PD-L1 expression was evaluated as a possible biomarker and correlated with a higher response rate [39]. Nevertheless, monotherapy with checkpoint-inhibitors showed only modest anti-tumoral activity in recurrent ovarian cancer. More promising results were reported in the phase-II-trials MEDIOLA and TOPACIO. Combination of checkpoint- and PARP-inhibitors in recurrent ovarian cancer resulted in ORR of 18 to 72% and DCR of 65% [40,41]. Consequently, ongoing phase-III-trials frequently combine checkpoint-inhibitors with standard chemotherapy or PARP-inhibitors. Currently (accessed on 23 February 2022) there are fourteen phase-III-trials registered on clinicaltrials.gov which are evaluating checkpoint-inhibitors in ovarian cancer (six in primary and eight in recurrent disease). In Phase I and II more than 100 trials can be found. This demonstrates the future potential and importance of understanding the immune infiltrate and checkpoint-inhibitor based therapy in ovarian cancer.

1.2.4. Intratumoral Heterogeneity of Microenvironment and Immune Infiltrate

As it can be seen in Table 1, more than 70% of the patients suffer from tumor spread outside the pelvis at initial diagnosis, mostly in form of peritoneal and omental metastases. Due to the different immunological structure of peritoneal and omental tissues, differences in the tumor microenvironment can be expected. Intratumoral heterogeneity has already been observed on genomic, transcriptomic, and proteomic level [42,43]. Considering the different composition of peritoneal and omental tissues [27,44], various tumor microenvironments can be expected. Heindl et al. showed a correlation of high intratumoral diversity of the tumor microenvironment composition and poor prognosis [45]. Jimenez-Sanchez et al. could prove heterogeneity between the immune infiltrate of multiple tumor lesions of one patient. Further different phenotypes were associated with stable or progressing metastases [46]. Considering the increasing relevance of immunotherapy, immune heterogeneity must be considered as a further potential prognostic and predictive factor in ovarian cancer.

1.3. Biomarker Expression in Ovarian Cancer

1.3.1. Importance of Biomarkers for Patient Stratification

Biomarkers are defined as a “characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions.” They can “include molecular, histologic, radiographic, or physiologic characteristics”. [47] Defined by the process that is measured, there are various categories of biomarkers.

Category	Description
Susceptibility/ risk	Indicates potential for developing a disease
Diagnostic	Detects or confirms presence of a disease
Monitoring	Detects a change in the degree or extent of disease over time
Prognostic	Identifies likelihood of a clinical event, disease recurrence or progression
Predictive	Identifies individuals, who will experience effect from exposure to treatment

Table 2: Categories of Biomarkers (according to [47,48])

As already mentioned above, due to poor prognosis and early relapse, there is an urgent need for prognostic and predictive biomarkers in primary ovarian cancer. They could improve patient stratification and therefore enable treatment planning for individual patients. There are some features of tumor biology, which have already proven to be prognostic biomarkers (see 1.1.4) and help to assess the course of disease in individual patients. Identification of new negative prognostic biomarkers, which can be addressed by targeted therapy, could expand treatment options.

High rates of relapse and platinum-resistance in ovarian cancer have triggered a search for reliable predictive biomarkers. Many candidates for prediction of platinum-sensitivity have been found in the past [49,50], but none has proven its predictive value in large trials and been established in guidelines. Once platinum-resistance is proven, there are only a few therapy options with small chance of long-term response. Consequently, more targeted therapies must be established for patients with platinum-resistance.

The aim of the following publications was the identification of prognostic and predictive biomarkers, that can be detected by immunohistochemistry and addressed by existing targeted therapies.

1.3.2.Integrins in Ovarian Cancer

Integrins are transmembrane cell-adhesion receptors. They are heterodimers built from one α-subunit and one β-subunit. Currently, there are 18 α -subunits and 8 β-subunit identified in mammals, which can form 24 different heterodimers. As they are transmembrane proteins, they connect the cytoskeleton to the ECM and are capable of bidirectional signaling. Changes in the extracellular milieu can be detected and influence intracellular processes. Otherwise, intracellular signaling can change allosteric conformation of integrins and therefore their affinity to ligands [51,52]. Due to these features, various steps of cancer progression are mediated by integrins. As main cell-adhesion-receptors they are crucial for migration, invasion, and metastasis [53,54]. As already mentioned above, ovarian cancer metastasis predominantly takes part in the peritoneal cavity. Cells detach from the primary tumor and spread in the peritoneal fluid. Final attachment to mesothelial

cells of peritoneal and omental tissue, local invasion and proliferation is mediated by integrins [25,55,56]. Consequently, they are crucial for interactions with the tumor microenvironment [26,27]. Previous studies indicate the ability of integrins in promoting chemotherapy resistance in ovarian cancer. These cell-adhesion mediated drug resistance (CAM-DR) is based on intracellular signaling initiated by integrin-binding to the ECM [57,58].

Especially β1-integrins are involved in the above-mentioned processes in ovarian cancer. Integrin α4β1 and α5β1 are known to play a major role in attachment of cancer cells to the mesothelial cells of peritoneal and omental tissue [56,59]. Less is known about the heterodimer α2β1, but previous studies suggest a contribution to chemotherapy resistance in primary ovarian cancer [60,61].

Being an essential part of progression and metastasis in ovarian cancer, integrins are promising candidates as prognostic and predictive biomarkers, but also as targets for development of further therapeutic strategies in ovarian cancer.

1.3.3.Integrin-targeted Therapy

Due to their involvement in a wide range of cellular processes and functions integrins are promising targets for new treatment strategies concerning different pathologies. Several drugs have already been approved: αIIbβ3-antagonists are used for the treatment of myocardial infarction, Natalizumab and Vedolizumab (antibodies against integrin α4β1 and α4β7) are approved for treating inflammatory bowel diseases and multiple sclerosis [62]. Although integrin-targeted therapy in cancer has been subject of extensive research in the past two decades, there has been no approval until now. Especially inhibitors for integrin αvβ3, which is known to play an important role in tumor angiogenesis, showed promising results in preclinical data. Cilengitide, a cyclic pentapeptide, was administered to glioblastoma patients even in a phase-III-trial, but finally failed for a positive outcome [63,64].

Failure due to intensive research in the last decades can be explained by the complex biology of integrins. After binding to a ligand, integrins perform conformational changes, which affect the affinity to the ligand or the intracellular activity. Therefore, antibodies or peptides, which are actually designed for inhibition of integrin activity, can have the opposite effect. Another point is the reduced selectivity of designed ligands. Mostly they are directed towards one subunit, which results in binding to all heterodimers including this subunit [62].

There are new approaches, directing these problems. Allosteric ligands which stabilize the inactive state of an integrin are designed. Further, especially tumor entities or single patients with high expression of a single integrin can be targeted by an integrin-binding-partner, which is conjugated with a cytotoxic molecule [62,65]. Therefore, identifying expression levels of integrin in ovarian cancer might specify the suitability as a possible therapeutic target.

1.4. Objectives

As shown above, patients with ovarian cancer suffer from poor prognosis. Despite radical surgical resection and adjuvant chemotherapy, 70 to 80% of patients experience relapse. There is an urgent need for prognostic and predictive biomarkers, which enable patient stratification. They could help to identify patients, who benefit from platinum-based chemotherapy and further represent possible drugable targets for new therapy options in ovarian cancer.

The aim of the following publications was to find prognostic and predictive biomarkers in the tumor microenvironment of ovarian cancer. Expression of possible candidates was analyzed via immunohistochemical staining and correlated with clinicopathological data.

As one crucial component of the tumor microenvironment, the immune infiltrate represented the main topic of the first publication. It evaluates the composition of the immune infiltrate, its predictive value and intratumoral heterogeneity between corresponding lesions within the peritoneal cavity.

The second publication focused on the cell adhesion molecule integrin α2β1. The prognostic and predictive value of the single integrin expression, but also of dual expression with other biomarkers has been evaluated. In addition to the first publication, integrin α2β1 expression was correlated with the immune infiltrate.

1.5. Contribution

For both publications, patients and their clinicopathological data from the SpheroID-study were included. This study was initiated by PD Barbara Mayer and approved by the institutional review board of the Ludwig-Maximilians-Universität, Munich, Germany at 07th August 2012 (No.278/04).

Experimental design of both studies was developed by the authors Katharina Dötzer and PD Barbara Mayer.

In the first publication the author contributed to the development of a new immune cell counting method (QTiS algorithm) [66]. She conducted the immunohistochemical staining, photodocumentation of the results and the application of the new-established QTiS algorithm. The statistical analysis, including the correlation of experimental data with clinicopathological data and survival analysis as well as the comparison of primary tumor and corresponding lesions, was performed by the author under the supervision of Alexander Crispin. She prepared the final manuscript, including all tables and figures under the supervision of Barbara Mayer.

For the second publication Katharina Dötzer conducted the immunohistochemical staining with the help of Michael Pohr, Anton Stolp and Frank Arnold. She evaluated the biomarker expression by semiquantitative scoring. The statistical analysis, including the correlation of experimental and clinicopathological data as well as uni- and multivariate survival analysis was performed by the author. The manuscript, including tables and figures, was prepared by Katharina Dötzer under the supervision of Barbara Mayer.

2. Summary

2.1. Abstract (English)

Despite intensive research in recent years, diagnosis of ovarian cancer is still connected to a poor prognosis. Due to advanced tumor stage at the time of diagnosis, most of patients must undergo radical surgery followed by platinum-based chemotherapy. 70 to 80% of patients suffer from relapse in spite of this intensive treatment. To identify patients, who do not respond to platinum-based therapy, additional prognostic and predictive biomarkers must be found. They can enable patient stratification and further represent targets for new therapy options. Due to fast tumor spread within the peritoneal cavity, ovarian cancer cells are interacting with different cell types of the tumor microenvironment from early stages on. Addressing this key feature, prognostic and predictive markers were searched in the tumor microenvironment of ovarian cancer. For this purpose, tumor samples were analyzed immunohistochemically and correlated with clinicopathological data of patients.

The publication “Immune Heterogeneity Between Primary Tumors and Corresponding Metastatic Lesions and Response to Platinum Therapy in Primary Ovarian Cancer” analyzed differences in the immune infiltrate of primary tumors and corresponding omental and peritoneal lesions, as well as the associated predictive impact.

Immune heterogeneity was observed between omental lesions and corresponding primary tumors. A higher count of stromal $CD45^+$ ($p = 0.007$), $CD3^+$ ($p = 0.005$), $CD8^+$ ($p = 0.012$) und $PD\text{-}1^+$ ($p = 0.013$) cells was found in omental lesions. Furthermore, lymph node metastasis correlated with a higher stromal infiltrate of $CD45^+$ ($p = 0.018$) and $CD3^+$ ($p = 0.037$) cells in omental lesions.

Intratumoral heterogeneity was also found between primary tumors and corresponding peritoneal lesions. While more $PD\text{-}1^+$ immune cells were detected in primary tumors ($p = 0.054$), PD-L1 expression was trending to be higher in peritoneal lesions ($p = 0.078$). Additionally, tumor heterogeneity between primary tumors and their peritoneal lesions was identified as a predictive marker for platinum-sensitivity. In case of a higher amount of intratumoral $CD8^+$ cells in the peritoneal lesion compared to the primary tumor, platinum-sensitivity was more frequent ($p = 0.045$). On the contrary, a higher stromal infiltrate of $PD\text{-}1^+$ cells in the peritoneal lesion was associated with reduced platinum-sensitivity ($p = 0.045$).

In addition to the composition of the tumor microenvironment, mediators of interaction between cancer cells and the tumor microenvironment were analyzed in the publication “Integrin α2β1 Represents a Prognostic and Predictive Biomarker in Primary Ovarian Cancer”.

High expression of the cell-adhesion-molecule integrin α2β1 was an independent prognostic factor for shorter PFS ($p = 0.021$) and PFI ($p = 0.022$). Relations to other therapy-relevant biomarkers have been evaluated. A significant correlation was found between high expression of integrin α2β1 with EGFR ($p = 0.027$) and ERα ($p = 0.035$). Further, dual expression of Integrin α2β1 with HGFR (PFS/PFI: $p = 0.004$) or CD44v6 (PFS: $p = 0.000$; PFI: $p = 0.001$; OS: $p = 0.025$) is associated with poor survival.

A reference to the first publication was made by evaluation of the immune infiltrate in correlation to integrin α2β1 expression. Low counts of various cell types of the immune infiltrate were associated with a high expression of Integrin α2β1 (CD3 intratumoral: p = 0.017; CD3 stromal: p = 0.034; PD-1 intratumoral: p = 0.002; PD-1 stromal: p = 0.049). This confirms integrin α2β1 as a poor prognostic factor and outlines its possible immunosuppressive role.

In search for additional therapeutic strategies to treat ovarian cancer more attention must be paid to the tumor microenvironment. The expression of integrin α2β1 and the the heterogeneity of the immune infiltrate have proven to be predictive markers for platinum sensitivity. Therefore, they may represent possible stratification markers for future therapy choices. In addition, the findings can contribute to a closer understanding of platinum resistance in ovarian cancer.

The above identified prognostic and predictive biomarkers can help to personalize treatment choices and identify patients fitting for platinum-based chemotherapy, immunotherapy, like checkpoint-inhibitors, or new targeted therapies. Due to frequent co-expression and to evade therapy resistance dual-targeting must be considered as promising approach.

2.2. Zusammenfassung (Deutsch)

Trotz intensiver Forschung in den letzten Jahren ist die Prognose für Patienten mit der Diagnose Ovarialkarzinom weiterhin schlecht. Aufgrund meist später Diagnosestellung in einem fortgeschrittenen Tumorstadium müssen sich die meisten Patienten einer ausgedehnten Operation mit anschließender platinhaltiger Chemotherapie unterziehen. Bei 70 bis 80% der Patienten kommt es trotz dieser intensiven Therapie zum Rezidiv. Daher besteht ein hoher Bedarf an prognostischen und prädiktiven Biomarkern, welche Patienten erfassen, die schlecht auf eine platin-basierte Chemotherapie ansprechen. Diese Biomarker können sowohl bei der Einteilung der Patienten nach Prognose helfen als auch potenzielle Ziele für neue Therapieoptionen darstellen. Da sich das Ovarialkarzinom meist früh in der Peritonealhöhle ausbreitet, interagieren die Tumorzellen der Metastasen mit sehr unterschiedlichen Formen von Mikromilieu. Hier kann auf der Suche nach neuen prognostischen und prädiktiven Markern angesetzt werden. Zu diesem Zweck erfolgte die immunhistochemische Analyse verschiedener Tumorproben und die anschließende Korrelation mit klinischen und pathologischen Daten der entsprechenden Patienten.

Die Veröffentlichung „Immune Heterogeneity Between Primary Tumors and Corresponding Metastatic Lesions and Response to Platinum Therapy in Primary Ovarian Cancer“ beschreibt die Unterschiede des Immuninfiltrats im Primärtumor und in den zugehörigen Metastasen des Omentums und Peritoneums. Diese Unterschiede erweisen sich als mögliche prädiktive Faktoren.

Es zeigten sich deutliche Unterschiede in der Zusammensetzung der Immunzellen zwischen dem Primärtumor und den Metastasen des Omentums. Im Omentum konnten deutlich mehr $CD45^+$ ($p = 0.007$), $CD3^+$ ($p = 0.005$), $CD8^+$ ($p = 0.012$) und $PD\text{-}1^+$ ($p = 0.013$) Zellen im Stroma beobachtet werden. Zudem fanden sich bei Patientinnen mit bereits bestehender Lymphknotenmetastasierung mehr stromale $CD45^+$ ($p = 0.018$) and $CD3^+$ ($p = 0.037$) Zellen im Omentum.

Unterschiede zeigten sich auch zwischen dem Primärtumor und den entsprechenden peritonealen Metastasen. Es fanden sich mehr PD-1$^+$ Immunzellen im Primärtumor ($p = 0.054$), dafür war die PD-L1 Expression der Tumorzellen in den Metastasen des Peritoneums höher ($p = 0.078$). Diese Heterogenität zwischen Primärturmor und peritonealen Metastasen stellte sich als möglicher prädiktiver Marker für Platinsensitivität heraus. Falls sich intratumoral in den peritonealen Metastasen mehr CD8$^+$ Zellen als im Primärtumor fanden, zeigten sich die Karzinome häufiger platinsensitiv ($p = 0.045$). Ein ausgeprägteres stromales PD-1$^+$ Infiltrat der peritonealen Metastase war hingehen mit einer reduzierten Platinsensitivität assoziiert ($p = 0.045$).

Neben der Zusammensetzung des Mikromilieus wurden in der Veröffentlichung „Integrin α2β1 Represents a Prognostic and Predictive Biomarker in Primary Ovarian Cancer“ auch das Zusammenspiel zwischen Mikromilieu und Krebszellen untersucht.

Eine hohe Expression des Zelladhäsionsmolekül Integrin α2β1 zeigte sich als unabhängiger prognostischer Faktor für ein kürzeres PFS ($p = 0.021$) und PFI ($p = 0.022$). Zudem ergaben sich auch Zusammenhänge bezüglich anderer therapierelevanter Biomarker. Es zeigte sich eine hohe Expression von EGFR ($p = 0.027$) oder ERα ($p = 0.035$) bei gleichzeitiger hoher Expression von Integrin α2β1. Eine gleichzeitige hohe Expression von Integrin α2β1 und HGFR (PFS/PFI: $p = 0.004$) oder CD44v6 (PFS: $p = 0.000$; PFI: $p = 0.001$; OS: $p = 0.025$) war mit schlechterem Überleben assoziiert.

Ein Bezug zur ersten Veröffentlichung wurde hergestellt, indem ein möglicher Zusammenhang zwischen der Expression von Integrin α2β1 und Immunzellen untersucht wurde. Tumoren mit geringerer Anzahl verschiedener Leukozyten (CD3 intratumoral: $p = 0.017$; CD3 stromal: $p = 0.034$; PD-1 intratumoral: $p = 0.002$; PD-1 stromal: $p = 0.049$) zeigten gleichzeitig häufig eine hohe Integrin α2β1 Expression. Dies bestätigt nochmals Integrin α2β1 als negativen prognostischen Faktor und unterstreicht seine immunsuppressive Wirkung.

Auf der Suche nach neuen Therapiemöglichkeiten für das Ovarialkarzinom muss der Fokus in Zukunft auf das Mikromilieu des Tumors gelegt werden. Sowohl die Expression von Integrin α2β1 als auch die Heterogenität der Immunzellen zeigten sich als prädiktive Faktoren zur Vorhersage von Platinsensitivität. Somit eignen sie sich sowohl als Marker für weitere Therapieentscheidungen, als auch als möglicher Ausgangspunkt um die Mechanismen der Platinresistenz beim Ovarialkarzinom zu erforschen.

Die genannten neuen prognostischen und prädiktiven Biomarker können in Zukunft helfen, Therapieentscheidungen zu personalisieren und die richtige Wahl aus platinbasierter Chemotherapie, Immuntherapie oder neuen zielgerichteten Therapien für jeden Patienten zu treffen. Um Therapieresistenzen zu vermeiden, müssen aufgrund von häufig gleichzeitiger Expression der entsprechenden Marker auch Kombinationstherapien in Erwägung gezogen werden.

cancers

MDPI

Article

Immune Heterogeneity Between Primary Tumors and Corresponding Metastatic Lesions and Response to Platinum Therapy in Primary Ovarian Cancer

Katharina Dötzer [1], Friederike Schlüter [1], Markus Bo Schoenberg [1], Alexandr V. Bazhin [1,2], Franz Edler von Koch [3], Andreas Schnelzer [4], Sabine Anthuber [5], Dieter Grab [6], Bastian Czogalla [7], Alexander Burges [7], Jens Werner [1,2], Sven Mahner [7] and Barbara Mayer [1,2,*]

1 Department of General, Visceral and Transplant Surgery, Ludwig-Maximilians-University Munich, Marchioninistraße 15, 81377 Munich, Germany
2 German Cancer Consortium (DKTK), Partner Site Munich, Pettenkoferstraße 8a, 80336 Munich, Germany
3 Department of Obstetrics and Gynecology, Klinikum Dritter Orden, Menzinger Straße 44, 80638 Munich, Germany
4 Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technical University Munich, Ismaninger Straße 22, 81675 Munich, Germany
5 Department of Obstetrics and Gynecology, Clinic Starnberg, Oßwaldstraße 1, 82319 Starnberg, Germany
6 Department of Obstetrics and Gynecology, Clinic Harlaching, Sanatoriumsplatz 2, 81545 Munich, Germany
7 Department of Obstetrics and Gynecology, University Hospital, Ludwig-Maximilians-University Munich, Marchioninistraße 15, 81377 Munich, Germany
* Correspondence: barbara.mayer@med.uni-muenchen.de; Tel.: +49-89-4400-76438

Received: 4 August 2019; Accepted: 14 August 2019; Published: 26 August 2019

Abstract: $CD3^+$ and $CD8^+$ lymphocytes are well known prognostic markers in primary ovarian cancer. In contrast, the predictive value of the immune infiltrate concerning treatment response and the involvement of immune heterogeneity between primary and metastatic lesions are poorly understood. In this study, the immune infiltrate of 49 primary tumors and 38 corresponding lesions in the omentum (n = 23) and the peritoneum (n = 15) was immunohistochemically analyzed and correlated with clinicopathological factors and platinum-sensitivity. Immune heterogeneity was observed between paired primary and metastatic lesions for all immune cell phenotypes. The stromal immune infiltrate was higher in the omental lesions than in the primary tumors, which was reflected by CD45 (p = 0.007), CD3 (p = 0.005), CD8 (p = 0.012), and PD-1 (programmed cell-death protein 1) (p = 0.013). A higher stromal infiltrate of both $CD45^+$ and $CD3^+$ cells in the omental lesions was associated with the detection of lymph node metastasis (CD45, p = 0.018; CD3, p = 0.037). Platinum-sensitive ovarian cancers revealed a higher intratumoral $CD8^+$ infiltrate in the peritoneal lesions compared to the primary tumors (p = 0.045). In contrast, higher counts of stromal $PD\text{-}1^+$ cells in the peritoneal lesions have been associated with reduced platinum-sensitivity (p = 0.045). Immune heterogeneity was associated with platinum response and might represent a selection marker for personalized therapy.

Keywords: ovarian cancer; metastatic lesions; tumor microenvironment; TILs; immune heterogeneity; platinum-sensitivity; immune checkpoints

1. Introduction

High cell counts of various immune cell markers in ovarian cancer have been identified as positive prognostic factors. An especially high intratumoral infiltrate of $CD3^+$ and $CD8^+$ cells is associated with increased progression free survival (PFS) and overall survival (OS). This has been investigated in several studies [1,2]. Whereas the prognostic value of immune infiltrate in ovarian cancer has

been evaluated in detail, there have been less studies investigating the predictive value for treatment response. More than two-thirds of the patients experience relapse within the first three years, despite optimal surgery and adjuvant chemotherapy with carboplatin and paclitaxel [3].

Incorporation of biological drugs may improve PFS [4,5]. Tumor biology has an increasing impact in the treatment of recurrent ovarian cancer, extending the classification according to time to relapse after chemotherapy: platinum-resistant (<6 months), partially platinum-sensitive (6–12 months), and platinum-sensitive (>12 months) [6,7]. This clinical classification has been used when choosing second-line chemotherapy [8]. Further stratification factors are required for specifying first-line therapy [9]. Evaluation of the impact of the immune infiltrate on chemosensitivity might help to select the most appropriate patients for treatment with chemotherapy. In addition, the immune infiltrate might be a predictive marker for immunotherapy.

As a result of the discovery of lymphocytes as an important factor in the antitumoral defence of ovarian cancer, checkpoint inhibitors have rapidly emerged in past years in new therapeutic approaches [10–13]. Recently, various studies about treatment with checkpoint inhibitors in combination with platinum-based chemotherapy have started, investigating both primary ovarian cancer (NCT02718417, NCT02520154, NCT02766582) and the recurrent situation (NCT02891824) [14]. As none of these trials considers specific biomarkers to preselect appropriate patients, there is an obvious need to investigate the immune infiltrate in primary tumors. Because most ovarian cancer patients are diagnosed in an advanced stage [15], metastatic lesions in the peritoneum and omentum need to be analyzed in addition. While genomic, transcriptomic, and proteomic intratumoral heterogeneity are well published in ovarian cancer [16–19], immune heterogeneity has not been systematically investigated.

In the present study, various immune cell phenotypes were analyzed in the primary tumor and compared with metastatic lesions in the peritoneum and the omentum. In detail, the density of $CD45^+$, $CD3^+$, $CD8^+$, PD-1^+ (programmed cell-death protein 1), and PD-$L1^+$ (programmed cell-death ligand 1) cells was evaluated in the stromal and intratumoral areas of the different lesions and correlated with a number of clinicopathological factors and the platinum-sensitivity. Heterogenous distribution of the immune infiltrate might impact treatment management.

2. Results

2.1. Patient Characteristics

Clinicopathological characteristics are shown in Table 1. Most patients presented with high-grade, serous ovarian carcinoma and have been diagnosed in an advanced FIGO (International Federation of Gynaecology and Obstetrics) stage, with the presence of ascites and lymphatic vessel invasion. Seventy-one percent of all patients had a complete surgical resection of all macroscopic visible tumor. In accordance with the advanced tumor stage, 82% of the patients received a carboplatin–paclitaxel-based chemotherapy. The median PFS was 19 months (range 9–42). Concerning time to relapse after chemotherapy, 14 patients were defined as reduced platinum-sensitive (tumor relapsed ≤12 months after chemotherapy) and 28 patients as full platinum-sensitive (tumor relapsed >12 months after chemotherapy).

The presence of distant metastases at time of diagnosis was significantly associated with reduced platinum-sensitivity ($p = 0.015$, Table S1). The presence of ascites before surgery ($p = 0.083$) and macroscopic residual tumor after surgery ($p = 0.067$) showed a trend to reduced platinum-sensitivity.

In accordance with these results, the presence of metastases ($p_{log\text{-}rank} = 0.031$, $p_{Breslow} = 0.035$), macroscopic residual tumor after surgery ($p_{log\text{-}rank} = 0.01$, $p_{Breslow} = 0.005$), and vascular invasion ($p_{log\text{-}rank} = 0.006$, $p_{Breslow} = 0.03$) correlated significantly with shorter PFS (Figure S1).

Table 1. Patient characteristics.

		n or Value	%
Age	mean/median range	62/66 years 24–83 years	
FIGO Stage	I/II III IV	0 35 14	0.0 71.4 28.6
pT	pT2 pT3	5 44	10.2 89.8
pN	pN0 pN1 Nx	6 32 11	12.2 65.3 22.4
cM	cM0 cM1	35 14	71.4 28.6
Primary Tumor Site	Ovarian Fallopian Tube Peritoneal	39 7 3	79.6 14.3 6.1
Histological Subtype	Serous Other	44 5	89.8 10.2
Grading	G1/G2 G3	1 47	4.0 95.9
Ascites	yes no	41 8	83.7 16.3
Macroscopic Residual Tumor after Surgery	None <1 cm >1 cm	35 8 6	71.4 16.3 12.2
Lymphatic Vessel Invasion	yes no missing	26 21 2	53.1 42.9 4.1
Vascular Invasion	yes no missing	9 38 2	18.4 77.6 4.1
First-Line-Treatment	C C+P C+P+B None	5 15 25 4	10.2 30.6 51.0 8.2
Relapse after Chemotherapy	< 6 months 6–12 months >12 months none or non-sufficient chemotherapy	2 12 28 7	4.1 24.5 57.1 14.3

n: number of patients, FIGO: International Federation of Gynaecology and Obstetrics, p: pathological, c: clinical, T: extent of primary tumor, N: regional lymph node metastasis, Nx: no evaluation of lymph node status, M: distant metastasis, C: Carboplatin, P: Paclitaxel, B: Bevacizumab.

2.2. Immune Infiltrate in Primary Tumor

All immune cell phenotypes were detected in the stromal area of the primary tumor in a higher fraction compared to the intratumoral area. This finding was independent from the method of evaluation. The highest density was observed for $CD45^+$ cells, followed by $CD3^+$ cells, $CD8^+$ cells, and PD-1^+ cells (Table 2).

Table 2. Density and spatial distribution of immune cell phenotypes in different lesions of ovarian cancer.

		PT	OM	PE
Rating	**CD45 stromal**			
	Mode (Range)	3 (1–5)	3 (2–5)	3;4 (2–5)
	CD45 intratumoral			
	Mode (Range)	1 (0–3)	1 (0–3)	1 (0–3)
Cell Count	**CD3 stromal**			
	Mean (Range)	626 (5–2491)	1241 (202–4157)	851 (117–2766)
	CD3 intratumoral			
	Mean (Range)	201 (0–1134)	212 (0–569)	272 (0–985)
	CD8 stromal			
	Mean (Range)	318 (0–1049)	623 (83–1910)	364 (0–843)
	CD8 intratumoral			
	Mean (Range)	88 (0–716)	104 (0–636)	130 (0–494)
	PD-1 stromal			
	Mean (Range)	73 (0–404)	91 (0–335)	130 (0–601)
	PD-1 intratumoral			
	Mean (Range)	26 (0–191)	21 (0–83)	33 (0–145)
Expression	**PD-L1**			
	Median (Range)	1% (0–20%)	0.5% (0–20%)	3% (0–20%)

n: number of analyzed tumor samples, PT: Primary tumor, OM: Omental lesion, PE: Peritoneal lesion, PD-1: programmed cell-death protein 1, PD-L1: programmed cell-death ligand 1. To compare primary tumor and corresponding lesions, a Wilcoxon signed-rank test was performed. Results are given in Section 2.3.

Patients who showed characteristics of tumor progression, i.e., vascular invasion, the detection of distant metastasis and the presence of ascites, at the time of diagnosis showed a strong leukocyte infiltrate. A high density (rating ≥3) of $CD45^+$ leukocytes was frequently observed in the stromal area of primary tumors with distant metastasis (cM1, 93%, Table S2). Contrary, stromal $CD45^+$ cells were less frequently found in cancers without distant metastasis (cM0, 63%, $p = 0.042$). All patients with a strong (rating ≥2) $CD45^+$ intratumoral infiltrate in primary tumor were suffering from ascites ($p = 0.006$). Vascular invasion significantly correlated with a high (>73 counts/mm^2) density of $PD\text{-}1^+$ cells in the stromal area of the primary tumor ($p = 0.013$). PD-L1 positivity was found more often in primary tumors with distant metastasis (86%) compared to cancers without distant metastasis (51%, $p = 0.049$). A high intratumoral density (>88 counts/mm^2) of $CD8^+$ cells was predominantly observed in older patients (>62 years, 78%, $p = 0.037$).

Primary tumors with a high (>201 counts/mm^2) intratumoral $CD3^+$ cell density showed a trend to full platinum-sensitivity ($p = 0.057$, Table 3).

Table 3. Immune cell phenotypes of primary tumor and corresponding lesions in relation to platinum-sensitivity.

	PT					OM/PT					PE/PT				
			Platinum-Sensitivity *					Platinum-Sensitivity *					Platinum-Sensitivity *		
		n	Red	Full	p #		n	Red	Full	p #		n	Red	Full	p #
CD45 stromal		42			1		19			0.350		12			0.250
	Low		4	8		**OM ≤ PT**		2	7		**PE ≤ PT**		2	9	
	High		10	20		**OM > PT**		5	5		**PE > PT**		1	0	
CD45 intratumoral		42			1		19			1		12			1
	Low		8	15		**OM ≤ PT**		6	11		**PE ≤ PT**		3	8	
	High		6	13		**OM > PT**		1	1		**PE > PT**		0	1	
CD3 stromal		42			1		19			1		12			0.523
	Low		8	16		**OM ≤ PT**		1	3		**PE ≤ PT**		2	3	
	High		6	12		**OM > PT**		6	9		**PE > PT**		1	6	
CD3 intratumoral		42			0.057		19			0.633		12			1
	Low		11	13		**OM ≤ PT**		2	6		**PE ≤ PT**		2	4	
	High		3	15		**OM > PT**		5	6		**PE > PT**		1	5	
CD8 stromal		42			0.748		19			1		12			0.523
	Low		7	16		**OM ≤ PT**		2	3		**PE ≤ PT**		1	6	
	High		7	12		**OM > PT**		5	9		**PE > PT**		2	3	
CD8 intratumoral		42			1		19			0.656		12			**0.045**
	Low		9	18		**OM ≤ PT**		2	5		**PE ≤ PT**		3	2	
	High		5	10		**OM > PT**		5	7		**PE > PT**		0	7	
PD-1 stromal		42			0.283		19			1		12			**0.045**
	Low		12	19		**OM ≤ PT**		2	4		**PE ≤ PT**		0	7	
	High		2	9		**OM > PT**		5	8		**PE > PT**		3	2	
PD-1 intratumoral		42			0.738		19			1		12			1
	Low		10	18		**OM ≤ PT**		5	8		**PE ≤ PT**		3	7	
	High		4	10		**OM > PT**		2	4		**PE > PT**		0	2	
PD-L1 Positivity		42			1		19			0.603		12			1
	No		5	10		**OM ≤ PT**		5	10		**PE ≤ PT**		2	7	
	Yes		9	18		**OM > PT**		2	2		**PE > PT**		1	2	

n: number of patients, red: reduced, PD-1: programmed cell-death protein 1, PD-L1: programmed cell-death ligand 1. * Platinum-sensitivity was defined as follows: reduced (relapse ≤ 12 months after chemotherapy) and full (relapse > 12 months after chemotherapy), # p-value calculated by Fisher's exact two-tailed test.

2.3. Immune Infiltrate in Metastatic Lesions

Direct comparison of primary tumors and omental lesions revealed a higher rating for stromal CD45$^+$ cells in the omental lesion in more than half of the patients (52%, $p = 0.007$, Table S3). Furthermore, in 16 cases (70%), the omental lesion showed a higher density of stromal CD3$^+$ and CD8$^+$ cells compared to the primary tumor ($p = 0.005$ and $p = 0.012$, Figure 1). Consequently, the mean count in omental lesions of stromal CD3$^+$ and CD8$^+$ cells was nearly two times higher. In addition, the majority of omental lesions (65%) revealed a higher infiltrate of stromal PD-1$^+$ cells ($p = 0.013$). There was no significant difference in intratumoral counts comparing primary tumors and omental lesions.

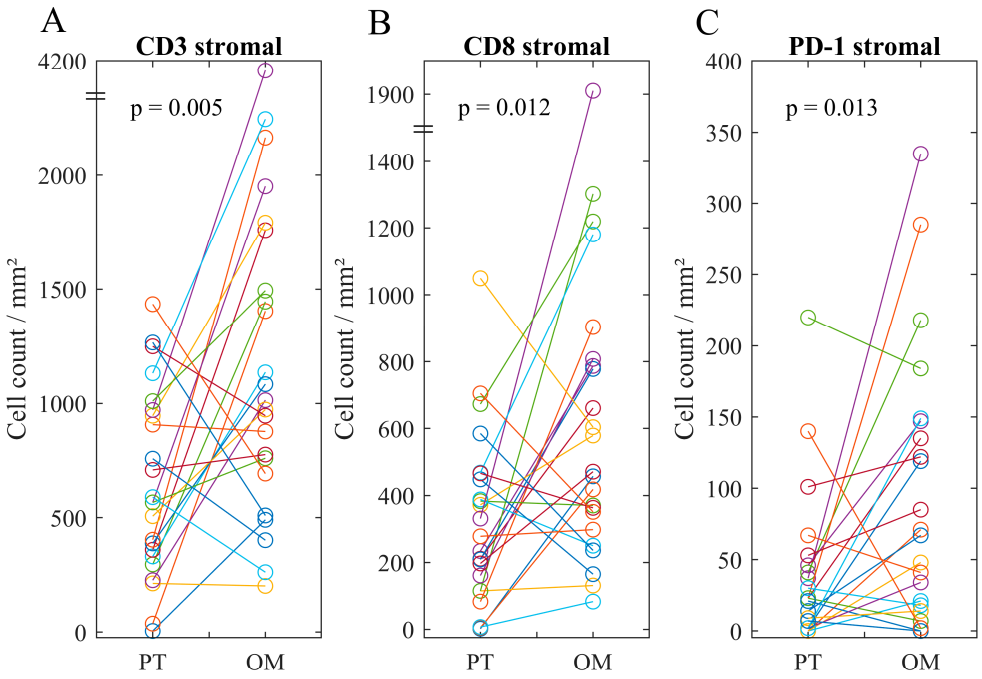

Figure 1. Scatter plots comparing immune cell phenotypes between primary tumor and the corresponding omental lesion. Counts of (**A**) CD3$^+$, (**B**) CD8$^+$, and (**C**) PD-1$^+$ (programmed cell-death protein 1) stromal cells. Counts of CD3$^+$, CD8$^+$, and PD-1$^+$ cells have been significantly higher in the omental lesions. *p*-value calculated by Wilcoxon signed-rank test.

In the stromal area of primary tumors and corresponding peritoneal lesions, no significant differences in the immune infiltrate could be found. In contrast, in ten cases (67%), the counts of intratumoral PD-1$^+$ cells were higher in the primary tumor compared to the corresponding peritoneal lesion ($p = 0.054$, Figure 2). Conversely, only one case (7%) showed a higher expression of PD-L1 in the primary tumor, while it was equal or lower in most patients (93%, $p = 0.074$).

2.4. Associations of Immune Heterogeneity

Patients with lymph node metastases revealed a strong stromal immune infiltrate in the omental lesion. Lymph node metastases have been found at the time of diagnosis in all patients with a higher immune infiltrate of stromal CD45$^+$ cells in the omental lesion compared to the primary tumor ($n = 11$, $p = 0.018$, Table S4). Most tumors with lymph node metastases (87%) revealed more stromal CD3$^+$ cells in the omental lesion than in the primary tumor ($p = 0.037$). No significant correlations have been found for peritoneal lesions (Table S5). Interestingly, immune heterogeneity between peritoneal lesions and primary tumors could be identified as a predictive marker. All patients with reduced platinum-sensitivity showed a stronger stromal infiltrate of PD-1$^+$ cells in the peritoneal lesion than in the primary tumor ($p = 0.045$, Table 3). In contrast, higher counts of intratumoral CD8$^+$ cells in the peritoneal lesions were associated with full platinum-sensitivity ($p = 0.045$).

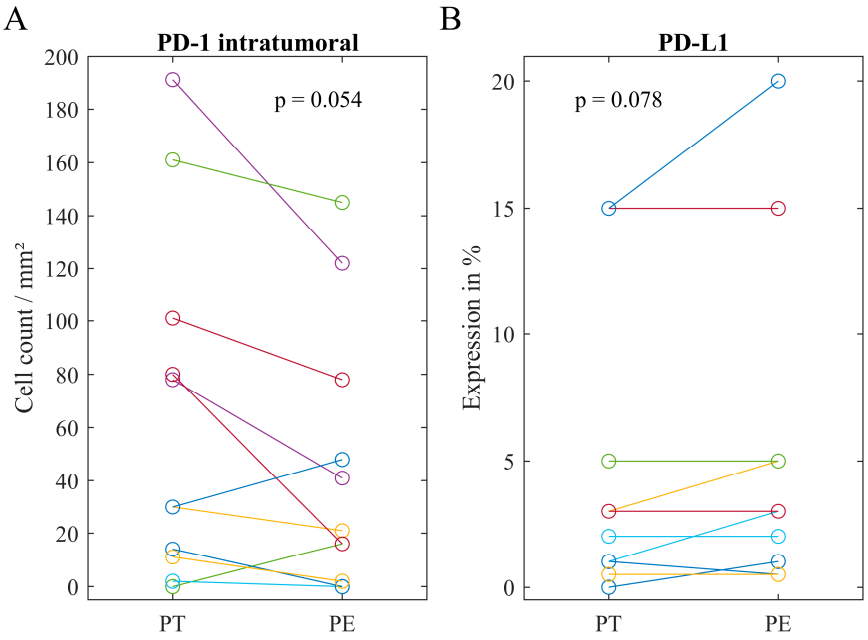

Figure 2. Scatter plots comparing immune cell phenotypes between primary tumor and the corresponding peritoneal lesion. (**A**) Counts of intratumoral PD-1$^+$ (programmed cell-death protein 1) cells have been in tendencies higher in primary tumor. (**B**) PD-L1 (programmed cell-death ligand 1) expression has been slightly higher in peritoneum. *p*-value calculated by Wilcoxon signed-rank test. Cases with the value '0' in primary tumor and peritoneum (A) n = 3 (B) n = 2 have been excluded from graph, but not from calculation.

3. Discussion

The urgent need for sustainable therapeutic strategies in ovarian cancer has triggered a comprehensive search for predictive biological markers. A number of new candidates have been identified in primary tumors [20]. In contrast, corresponding metastatic lesions are rarely analyzed although it is well known that the microenvironment has a profound impact on tumor cell biology and therapeutic response [21,22]. For the first time, in the present study, a systematic side-by-side comparison of the quantitative immune infiltrate in the primary tumor and metastatic lesions located in the omentum and peritoneum was performed and correlated with clinicopathological factors. In primary tumors, the presence of a strong CD45$^+$ infiltrate in both the stromal and intratumoral compartments correlated with parameters of advanced disease at time of initial diagnosis. In addition, strong PD-L1 expression in primary tumors correlated with metastatic disease. The presence of a strong stromal PD-1$^+$ infiltrate was frequently observed in primary tumors characterized by vascular invasion, suggesting an immunosuppressive role in advanced primary ovarian cancer. Indeed, a high expression of PD-1 and PD-L1 was reported to be associated with poor prognosis [23–25]. An increased intratumoral CD3$^+$ infiltrate in chemo-naive primary tumors was associated with response to platinum-based chemotherapy. This supports the 2011 study from Bösmüller et al., which indicated intratumoral lymphocyte density superior to ERCC-1 (excision repair cross-complementation group 1) expression to predict platinum response [26]. A number of different mechanisms is known to be involved in an increased antitumoral activity of lymphocytes induced by platinum-based chemotherapy [27,28]. Consequently, a pre-therapeutic increased number of CD3$^+$ cells might be associated with a higher efficacy. Methods to enhance this effect could be the application of dose-dense chemotherapy [29]. Stratification of primary tumors according to a strong CD3$^+$ infiltrate and a high PD-1/PD-L1 expression might identify ovarian cancer patients who are responsive to a combination of platinum-based chemotherapy with checkpoint-inhibitors [30,31].

In more than 70% of the patients, disease has already spread in peritoneal and omental tissue at the time of diagnosis, which is associated with an unfavorable prognosis [15,32]. A higher fraction of $CD45^+$, $CD3^+$, $CD8^+$, and $PD\text{-}1^+$ cells was observed in the stroma of omental lesions compared to the corresponding primary tumors. Higher counts of stromal $CD45^+$ and $CD3^+$ cells in the omental lesions were associated with the presence of lymph node metastases. Indeed, aggregates of leukocytes named milky spots are characteristic for the omental tissue and were found to promote immune suppression and, in consequence, facilitate tumor cell implantation [33,34]. They are discussed as a potential target for immunotherapy [35]. Combining strategies that enforce intratumoral recruitment of $CD8^+$ cells might, therefore, increase immunotherapeutic effects [36–38].

Similar to omental lesions, comparison between peritoneal lesions and their corresponding primary tumors identified changes in the immune cell infiltrate, i.e., less intratumoral $PD\text{-}1^+$ cells and a higher PD-L1 expression in the peritoneal metastases. Moreover, peritoneal lesions were characterized by a higher infiltration of intratumoral $CD8^+$ cells, which correlated with the response to the platinum-based first-line treatment. For example, in patient 5082, a high density ($30/mm^2$) of intratumoral $CD8^+$ cells was found in the peritoneal lesion, while there was a low intratumoral $CD8^+$ cells density ($7/mm^2$) in the corresponding primary tumor. In this individual patient platinum-based chemotherapy was successful, resulting in a relapse-free time of 26 months after chemotherapy. Conversely, in the peritoneal lesion of patient 5072, a low number ($2/mm^2$) of intratumoral $CD8^+$ cells was found, while a high infiltrate ($46/mm^2$) was found in the primary tumor. This patient suffered from a relapse within six months after platinum-based chemotherapy. Thus, for patients with a low peritoneal infiltrate, other therapeutic options than platinum-based chemotherapy should be considered right from the start. Treatment options might be non-platinum chemotherapeutic drugs or targeted therapies.

Conversely to the findings concerning intratumoral $CD8^+$ cells, a higher density of stromal $PD\text{-}1^+$ cells in the peritoneal lesions compared to the primary tumors was associated with a reduced platinum-sensitivity, which might be explained by impairment of these cells [23,24]. This further supports the close correlation between immune heterogeneity and platinum response. Comparing the immune contexture, defined as density, composition, and functionality of the immune infiltrate [39], in omental, peritoneal, and primary lesions, profound differences were observed. These changes might go along with dynamic variations in the tumor microenvironment at the different locations. In fact, the peritoneal cavity is characterized by complex multicellular interactions regulated by a network of multiple soluble factors [22,40,41].

Although the cohort of the present study can be described as representative and is comparable to cohorts of other clinical trials [42–44], the main limitation is the small number of patients. In particular, the number of samples of omental and peritoneal tissue was low. To confirm the results of the current pilot study, further analysis of immune heterogeneity should be pursued in a larger cohort. This study indicates that immune heterogeneity represents an important characteristic of ovarian cancer. Location-dependent changes in the immune cell infiltrate were identified as potential predictive markers for standard chemotherapy and immunotherapy. Thus, the systematic analysis of the immune contexture in both primary tumors and the corresponding metastatic lesions could have a relevant impact on treatment planning for individual cancer patients.

4. Patients and Methods

4.1. Study Population

Forty-nine patients diagnosed with a primary, chemo-naive ovarian, fallopian tube, or peritoneal cancer from the SpheroID-Study were included. Patients suffering from another neoplasia within the last five years were excluded. Informed consent was obtained from all patients. The study was approved by the institutional review board of the Ludwig-Maximilians-Universität Munich, Munich, Germany (No.278/04). Patients were recruited between September 2012 and January 2015 in five ovarian cancer centers, namely University Hospital, LMU Munich (n = 16), Klinikum Dritter Orden

(n = 16), Klinikum rechts der Isar, Technical University Munich (n = 7), Clinic Harlaching (n = 5), and Clinic Starnberg (n = 5). Standardized surgical resection and pathological analysis was conducted by the recruiting clinic. In 33 cases, in addition to primary tumor samples, corresponding omental or peritoneal lesions were resected. A detailed description of the tumor samples analyzed is given in Table S6. Patient-, tumor-, and treatment-related data for correlations was given in the routine reports and delivered in a pseudonymized form. Subsequently, for each clinicopathological parameter, patients were divided according to clinical relevance into two groups. For the parameter 'age', the mean value was chosen as cutoff. Analysis of platinum-sensitivity was performed after completion of chemotherapy [7,45]. Seven patients with no chemotherapy or a reduced number of treatment cycles (≤2) had to be excluded. In the present cohort, patients with platinum-resistant disease (n = 2) and patients with partially platinum-resistant disease (n = 12) were grouped and defined as reduced platinum-sensitive. This group was compared to the full platinum-sensitive group (n = 28) [6,7]. PFS was defined as the time from surgical treatment to the time of relapse or progression. As the median follow-up time was 26 months, OS has not been analyzed. Data from patients who did not die and had no relapse or progression was censored at the date of their last visit.

4.2. Immunohistochemistry

After surgical removal, tumor samples were snap frozen in liquid nitrogen. Serial cryosections (5 µm) were performed, fixed in acetone, and stained immunohistochemically using the avidin-biotin-peroxidase complex method [46]. Briefly, nonspecific binding Fc-regions were saturated with 10%-AB-Serum in phosphate-buffered-saline (Bio-Rad, Hercules, CA, USA, 805135) for 20 min. Endogenous biotin was blocked by the Avidin-/Biotin-Blocking-Kit (Vector Laboratories, Burlingame, CA, USA, SP-2001) according to the manufacturer. Primary antibodies were incubated for 60 min at room temperature, namely clone 2B11 + PD7/26 directed against CD45 (Dako, Santa Clara, CA, USA, M0701, working concentration [wc] 4.5 µg/mL), clone UCHT1 directed against CD3 (BD Biosciences, San Jose, CA, USA, 550368, wc 1.25 µg/mL), clone C8/144B directed against CD8 (Dako, M7103, wc 3 µg/mL), clone MIH4 directed against PD-1 (affymetrix eBiosciences, San Diego, CA, USA, 14-9969, wc 10 µg/mL) and clone MIH1 directed against PD-L1 (affymetrix eBiosciences, 14-5983, wc 10 µg/mL). The antibody Ber-EP4 directed against EpCAM (Dako, M0804, wc 2.5 µg/mL) was used to define tumor areas. The antibody MOPC21 (Sigma-Aldrich, St. Louis, MO, USA, M9269) was used as Immunoglobulin G1 isotype control in the relevant wc. Antibodies directed against CD45, CD3, and EpCAM were incubated with the secondary biotinylated antibody (Jackson ImmunoResearch, Cambridgeshire, U.K., 315-065-048, wc 0.75 µg/mL) for 30 min and, subsequently, with peroxidase-conjugated streptavidin (Jackson ImmunoResearch, 016-030-084, wc 1 µg/mL) for 30 min. Antibodies directed against CD8, PD-1, and PD-L1 were incubated with the ZytoChem Plus HRP-Kit (Zytomed Systems, Berlin, Germany, HRP060), as recommended by the manufacturer. Antigen–antibody reaction was visualized by 3-amin-9-ethylcarabazol (AEC, Sigma-Aldrich, A5754)-peroxide-solution for eight min in darkness. Sections were counterstained with Mayers hemalum solution (Merck, Darmstadt, Germany, 109249).

4.3. Semiquantitative Analysis of the $CD45^+$ Infiltrate

$CD45^+$ cells were detected as single cells and often in cell clusters of different size. This staining pattern could not be dissected precisely by the software ImageJ (Version 1.51h, National Institutes of Health, Bethesda, MD, U.S). Therefore, the CD45 staining was semiquantitatively rated in two different spatial areas (Table S7). In the intratumoral area, $CD45^+$ cells were found in direct contact with the cancer cells. In the stromal area adjacent to the tumor compartment, $CD45^+$ cells did not touch the tumor cells. Analysis was performed with the microscope (BX41, Olympus Corporation, Tokio, Japan).

4.4. Quantitative Analysis of Immune Cells

Quantitative analysis was performed for CD3, CD8, and PD-1. For each tissue section and each immune cell antigen, three separate regions characterized by an enriched infiltrate were selected,

separately for the stromal and the intratumoral area (defined previously and demonstrated in Figure 3). $CD8^+$ cells were counted in the same region as $CD3^+$ cells. For PD-1, known to be expressed on different cell types [47], enriched regions were evaluated independently from the CD3/CD8 area. A picture of each enriched region was captured (×200 magnification) using Zen 2.0 lite software (Carl Zeiss Inc., Oberkochen, Germany) with an AxioCam MRc5 camera (Carl Zeiss Inc., Oberkochen, Germany).

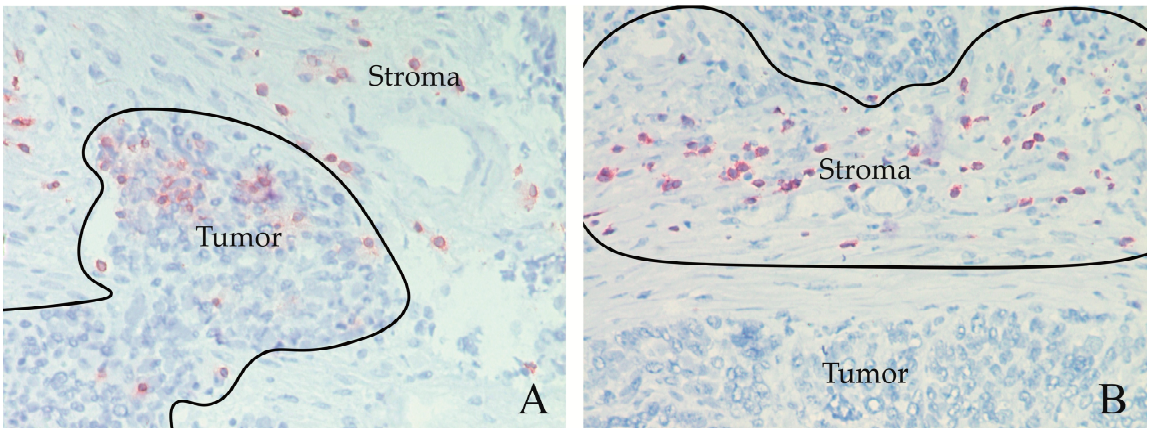

Figure 3. Immunohistochemical analysis demonstrating intratumoral (**A**) and stromal (**B**) $CD3^+$ cells. The black borders mark the enriched regions, in which $CD3^+$ cells were counted. Tumor and stroma areas are indicated. Magnification 200×.

Counting was done according to the QTiS algorithm by Miksch et al. [48–51] with the ImageJ software (Version 1.51h, National Institutes of Health, Bethesda, MD, USA). Briefly, after substraction of background, color deconvolution was performed, selecting example regions for the following spectra: background, hematoxylin, and AEC. In the AEC-spectrum an automatic threshold was applied to get a binary image. Watershed method was used to separate cell clusters. Cells were counted with the 'Analyze-Particle-Tool', considering the values of '700-infitiy' for size and '0.2–1.0' for circularity. The 'ROI-Manager' was used to distinguish between intratumoral and stromal area. For each tissue section, an average count of the three images that were taken was calculated. Cell counts were normalized to an average number of cells/mm^2.

The reliability of the semiquantitative rating system for $CD45^+$ cells was checked by correlation of ratings and counts of $CD3^+$ cells intratumoral and stromal. Spearman correlation coefficient was 0.803 ($p = 0.000$).

4.5. Semiquantitative Analysis of PD-L1

The fraction of all $PD\text{-}L1^+$ cells, including both cancer and immune cells in the intratumoral area, was estimated. PD-L1 positivity was defined as ≥1%. This approach has already been used in former clinical trials [52,53].

4.6. Statistical Analysis

Clinicopathological factors were grouped by clinical relevance. For the immune cell phenotypes CD3, CD8, and PD-1 identified in the respective spatial area the mean cell count was defined as cutoff to distinguish low infiltrates from high infiltrates. The cutoff for $CD45^+$ cells was chosen considering balanced group sizes (intratumoral: ≤1; >1; stromal: ≤2; >2). For comparison between corresponding location and primary tumor, the ratio between the cell counts was calculated for each immune cell phenotype in both spatial areas. To form two groups—'corresponding location lower/equal than primary tumor' and 'corresponding location higher than primary tumor'—≤1; >1 was chosen as the cutoff. The defined cutoffs were correlated with various clinicopathological data and platinum-sensitivity using the Fisher's exact two-tailed test. Cumulative survival probabilities were calculated by the Kaplan–Meier

method. Log-rank and Breslow tests were used to compare clinicopathological factors regarding PFS. Immune cell phenotypes between the primary tumor and different corresponding locations were compared by Wilcoxon signed-rank test. p-values < 0.050 were considered to be statistically significant; p-values < 0.100 have been reported. All statistical analyses were performed using SPSS Statistics (Version 23.0, IBM, Armonk, NY, USA).

5. Conclusions

Immunohistochemical comparison in ovarian cancer between primary tumors and the corresponding omental and peritoneal lesions demonstrated profound differences in the expression pattern of CD45, CD3, CD8, PD-1 and PD-L1. This immune heterogeneity made an impact on platinum-sensitivity. Variations in the immunologic tumor microenvironment might influence treatment selection for the individual ovarian cancer patient.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6694/11/9/1250/s1, Figure S1: Kaplan-Mayer-Curves for PFS of different clinicopathological parameters, Table S1: Clinicopathological factors in relation to platinum-sensitivity. Table S2: Immune cell phenotypes in primary tumor in relation to clinicopathological factors. Table S3: Comparison of the immune cell phenotypes between primary tumor and corresponding lesions. Table S4: Ratios of immune cell phenotypes between primary tumor and omental lesion in relation to clinicopathological factors. Table S5: Ratios of immune cell phenotypes between primary tumor and peritoneal lesion in relation to clinicopathological factors., Table S6: Description of primary tumor site and corresponding lesions per patient., Table S7: Description of rating method.

Author Contributions: Conceptualization, A.V.B., B.M.; Methodology, K.D., F.S., M.B.S., B.M.; validation, F.S., B.M.; formal analysis, K.D., B.M.; investigation, K.D.; resources, F.E.v.K., A.S., S.A., D.G., B.C., A.B., S.M.; writing—original draft preparation, K.D., B.M.; writing—review and editing, F.S., M.B.S., A.V.B., A.S., J.W., S.M.; visualization, K.D.; project administration, B.M.; funding acquisition, B.M.

Funding: This research was funded by the German Federal Ministry of Education and Research (Leading Edge Cluster m4) to B.M., Grant FKZ 16EX1021N.

Acknowledgments: The authors appreciate Dr. Alexander Crispin (Institute for Medical Information Processing, Biometry, and Epidemiology, Ludwig-Maximilians-University Munich) for statistical supervision and Maja Schorkowitz for acquisition of follow-up data.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Li, J.; Wang, J.; Chen, R.; Bai, Y.; Lu, X. The prognostic value of tumor-infiltrating T lymphocytes in ovarian cancer. *Oncotarget* **2017**, *8*, 15621–15631. [CrossRef] [PubMed]
2. OTTA Consortium. Dose-Response Association of CD8+ Tumor-Infiltrating Lymphocytes and Survival Time in High-Grade Serous Ovarian Cancer. *JAMA Oncol.* **2017**, *3*, e173290. [CrossRef] [PubMed]
3. Piccart, M.J.; Bertelsen, K.; James, K.; Cassidy, J.; Mangioni, C.; Simonsen, E.; Stuart, G.; Kaye, S.; Vergote, I.; Blom, R.; et al. Randomized Intergroup Trial of Cisplatin-Paclitaxel Versus Cisplatin-Cyclophosphamide in Women with Advanced Epithelial Ovarian Cancer: Three-Year Results. *J. Natl. Cancer Inst.* **2000**, *92*, 699–708. [CrossRef] [PubMed]
4. Rossi, L.; Verrico, M.; Zaccarelli, E.; Papa, A.; Colonna, M.; Strudel, M.; Vici, P.; Bianco, V.; Tomao, F. Bevacizumab in ovarian cancer: A critical review of phase III studies. *Oncotarget* **2017**, *8*, 12389–12405. [CrossRef]
5. Ledermann, J.; Harter, P.; Gourley, C.; Friedlander, M.; Vergote, I.; Rustin, G.; Scott, C.L.; Meier, W.; Shapira-Frommer, R.; Safra, T.; et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: A preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol.* **2014**, *15*, 852–861. [CrossRef]
6. Ledermann, J.A.; Raja, F.A.; Fotopoulou, C.; Gonzalez-Martin, A.; Colombo, N.; Sessa, C.; On behalf of the ESMO Guidelines Working Group. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **2013**, *24*, vi24–vi32. [CrossRef] [PubMed]

7. National Institute for Health and Care Excellence. Topotecan, Pegylated Liposomal Doxorubicin Hydrochloride, Paclitaxel, Trabectedin and Gemcitabine for Treating Recurrent Ovarian Cancer. Available online: https://www.nice.org.uk/guidance/ta389 (accessed on 3 June 2019).
8. Francis, J.; Coakley, N.; Elit, L.; Mackay, H.; The Gynecologic Cancer Disease Site Group. Systemic therapy for recurrent epithelial ovarian cancer: A clinical practice guideline. *Curr. Oncol.* **2017**, *24*, e540–e546. [CrossRef]
9. Colombo, N.; Sessa, C.; du Bois, A.; Ledermann, J.; McCluggage, W.G.; McNeish, I.; Morice, P.; Pignata, S.; Ray-Coquard, I.; Vergote, I.; et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: Pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. *Ann. Oncol.* **2019**, *30*, 672–705. [CrossRef]
10. Brahmer, J.R.; Tykodi, S.S.; Chow, L.Q.; Hwu, W.J.; Topalian, S.L.; Hwu, P.; Drake, C.G.; Camacho, L.H.; Kauh, J.; Odunsi, K.; et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N. Engl. J. Med.* **2012**, *366*, 2455–2465. [CrossRef]
11. Disis, M.L.; Patel, M.R.; Pant, S.; Hamilton, E.P.; Lockhart, A.C.; Kelly, K.; Beck, J.T.; Gordon, M.S.; Weiss, G.J.; Taylor, M.H.; et al. Avelumab (MSB0010718C; anti-PD-L1) in patients with recurrent/refractory ovarian cancer from the JAVELIN Solid Tumor phase Ib trial: Safety and clinical activity. *J. Clin. Oncol.* **2016**, *34*, 5533. [CrossRef]
12. Hamanishi, J.; Mandai, M.; Ikeda, T.; Minami, M.; Kawaguchi, A.; Murayama, T.; Kanai, M.; Mori, Y.; Matsumoto, S.; Chikuma, S.; et al. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients with Platinum-Resistant Ovarian Cancer. *J. Clin. Oncol.* **2015**, *33*, 4015–4022. [CrossRef] [PubMed]
13. Varga, A.; Piha-Paul, S.A.; Ott, P.A.; Mehnert, J.M.; Berton-Rigaud, D.; Johnson, E.A.; Cheng, J.D.; Yuan, S.; Rubin, E.H.; Matei, D.E. Antitumor activity and safety of pembrolizumab in patients (pts) with PD-L1 positive advanced ovarian cancer: Interim results from a phase Ib study. *J. Clin. Oncol.* **2015**, *33*, 5510. [CrossRef]
14. Gaillard, S.L.; Secord, A.A.; Monk, B. The role of immune checkpoint inhibition in the treatment of ovarian cancer. *Oncol. Res. Pract.* **2016**, *3*, 11. [CrossRef] [PubMed]
15. Torre, L.A.; Trabert, B.; DeSantis, C.E.; Miller, K.D.; Samimi, G.; Runowicz, C.D.; Gaudet, M.M.; Jemal, A.; Siegel, R.L. Ovarian cancer statistics, 2018. *CA A Cancer J. Clin.* **2018**, *68*, 284–296. [CrossRef] [PubMed]
16. Bashashati, A.; Ha, G.; Tone, A.; Ding, J.; Prentice, L.M.; Roth, A.; Rosner, J.; Shumansky, K.; Kalloger, S.; Senz, J.; et al. Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. *J. Pathol.* **2013**, *231*, 21–34. [CrossRef] [PubMed]
17. Choi, Y.J.; Rhee, J.K.; Hur, S.Y.; Kim, M.S.; Lee, S.H.; Chung, Y.J.; Kim, T.M.; Lee, S.H. Intraindividual genomic heterogeneity of high-grade serous carcinoma of the ovary and clinical utility of ascitic cancer cells for mutation profiling. *J. Pathol.* **2017**, *241*, 57–66. [CrossRef] [PubMed]
18. Hoogstraat, M.; de Pagter, M.S.; Cirkel, G.A.; van Roosmalen, M.J.; Harkins, T.T.; Duran, K.; Kreeftmeijer, J.; Renkens, I.; Witteveen, P.O.; Lee, C.C.; et al. Genomic and transcriptomic plasticity in treatment-naive ovarian cancer. *Genome Res.* **2014**, *24*, 200–211. [CrossRef]
19. Köbel, M.; Turbin, D.; Kalloger, S.E.; Gao, D.; Huntsman, D.G.; Gilks, C.B. Biomarker expression in pelvic high-grade serous carcinoma: Comparison of ovarian and omental sites. *Int. J. Gynecol. Pathol.* **2011**, *30*, 366–371. [CrossRef] [PubMed]
20. van Zyl, B.; Tang, D.; Bowden, N.A. Biomarkers of platinum resistance in ovarian cancer: What can we use to improve treatment. *Endocr.-Relat. Cancer* **2018**, *25*, R303–R318. [CrossRef]
21. Ghoneum, A.; Afify, H.; Salih, Z.; Kelly, M.; Said, N. Role of tumor microenvironment in ovarian cancer pathobiology. *Oncotarget* **2018**, *9*, 22832–22849. [CrossRef]
22. Worzfeld, T.; Pogge von Strandmann, E.; Huber, M.; Adhikary, T.; Wagner, U.; Reinartz, S.; Muller, R. The Unique Molecular and Cellular Microenvironment of Ovarian Cancer. *Front. Oncol.* **2017**, *7*, 24. [CrossRef] [PubMed]
23. Matsuzaki, J.; Gnjatic, S.; Mhawech-Fauceglia, P.; Beck, A.; Miller, A.; Tsuji, T.; Eppolito, C.; Qian, F.; Lele, S.; Shrikant, P.; et al. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 7875–7880. [CrossRef] [PubMed]
24. Wieser, V.; Gaugg, I.; Fleischer, M.; Shivalingaiah, G.; Wenzel, S.; Sprung, S.; Lax, S.F.; Zeimet, A.G.; Fiegl, H.; Marth, C. BRCA1/2 and TP53 mutation status associates with PD-1 and PD-L1 expression in ovarian cancer. *Oncotarget* **2018**, *9*, 17501–17511. [CrossRef] [PubMed]

25. Hamanishi, J.; Mandai, M.; Iwasaki, M.; Okazaki, T.; Tanaka, Y.; Yamaguchi, K.; Higuchi, T.; Yagi, H.; Takakura, K.; Minato, N.; et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 3360–3365. [CrossRef] [PubMed]
26. Bosmuller, H.; Haitchi-Petnehazy, S.; Webersinke, G.; Marschon, R.; Roithmeier, F.; Stummvoll, W.; Fehm, T.; Klier-Richter, M.; Bonzheim, I.; Staebler, A.; et al. Intratumoral lymphocyte density in serous ovarian carcinoma is superior to ERCC1 expression for predicting response to platinum-based therapy. *Virchows Arch.* **2011**, *459*, 183–191. [CrossRef] [PubMed]
27. de Biasi, A.R.; Villena-Vargas, J.; Adusumilli, P.S. Cisplatin-induced antitumor immunomodulation: A review of preclinical and clinical evidence. *Clin. Cancer Res.* **2014**, *20*, 5384–5391. [CrossRef] [PubMed]
28. Khairallah, A.S.; Genestie, C.; Auguste, A.; Leary, A. Impact of neoadjuvant chemotherapy on the immune microenvironment in advanced epithelial ovarian cancer: Prognostic and therapeutic implications. *Int. J. Cancer* **2018**, *143*, 8–15. [CrossRef]
29. Chang, C.L.; Hsu, Y.T.; Wu, C.C.; Lai, Y.Z.; Wang, C.; Yang, Y.C.; Wu, T.C.; Hung, C.F. Dose-dense chemotherapy improves mechanisms of antitumor immune response. *Cancer Res.* **2013**, *73*, 119–127. [CrossRef]
30. Chatterjee, J.; Dai, W.; Aziz, N.H.A.; Teo, P.Y.; Wahba, J.; Phelps, D.L.; Maine, C.J.; Whilding, L.M.; Dina, R.; Trevisan, G.; et al. Clinical Use of Programmed Cell Death-1 and Its Ligand Expression as Discriminatory and Predictive Markers in Ovarian Cancer. *Clin. Cancer Res.* **2017**, *23*, 3453–3460. [CrossRef]
31. Drakes, M.L.; Mehrotra, S.; Aldulescu, M.; Potkul, R.K.; Liu, Y.; Grisoli, A.; Joyce, C.; O'Brien, T.E.; Stack, M.S.; Stiff, P.J. Stratification of ovarian tumor pathology by expression of programmed cell death-1 (PD-1) and PD-ligand- 1 (PD-L1) in ovarian cancer. *J. Ovarian Res.* **2018**, *11*, 43. [CrossRef]
32. Heintz, A.P.; Odicino, F.; Maisonneuve, P.; Quinn, M.A.; Benedet, J.L.; Creasman, W.T.; Ngan, H.Y.; Pecorelli, S.; Beller, U. Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int. J. Gynaecol. Obstet.* **2006**, *95* (Suppl. 1), S161–S192. [CrossRef]
33. Meza-Perez, S.; Randall, T.D. Immunological Functions of the Omentum. *Trends Immunol.* **2017**, *38*, 526–536. [CrossRef] [PubMed]
34. Clark, R.; Krishnan, V.; Schoof, M.; Rodriguez, I.; Theriault, B.; Chekmareva, M.; Rinker-Schaeffer, C. Milky spots promote ovarian cancer metastatic colonization of peritoneal adipose in experimental models. *Am. J. Pathol.* **2013**, *183*, 576–591. [CrossRef] [PubMed]
35. McCloskey, C.W.; Rodriguez, G.M.; Galpin, K.J.C.; Vanderhyden, B.C. Ovarian Cancer Immunotherapy: Preclinical Models and Emerging Therapeutics. *Cancers* **2018**, *10*, 244. [CrossRef] [PubMed]
36. Wei, X.X.; Chan, S.; Kwek, S.; Lewis, J.; Dao, V.; Zhang, L.; Cooperberg, M.R.; Ryan, C.J.; Lin, A.M.; Friedlander, T.W.; et al. Systemic GM-CSF Recruits Effector T Cells into the Tumor Microenvironment in Localized Prostate Cancer. *Cancer Immunol. Res.* **2016**, *4*, 948–958. [CrossRef] [PubMed]
37. Halama, N.; Zoernig, I.; Berthel, A.; Kahlert, C.; Klupp, F.; Suarez-Carmona, M.; Suetterlin, T.; Brand, K.; Krauss, J.; Lasitschka, F.; et al. Tumoral Immune Cell Exploitation in Colorectal Cancer Metastases Can Be Targeted Effectively by Anti-CCR5 Therapy in Cancer Patients. *Cancer Cell* **2016**, *29*, 587–601. [CrossRef]
38. Zhang, S.; Zhong, M.; Wang, C.; Xu, Y.; Gao, W.Q.; Zhang, Y. CCL5-deficiency enhances intratumoral infiltration of CD8(+) T cells in colorectal cancer. *Cell Death Dis.* **2018**, *9*, 766. [CrossRef] [PubMed]
39. Fridman, W.H.; Zitvogel, L.; Sautes-Fridman, C.; Kroemer, G. The immune contexture in cancer prognosis and treatment. *Nat. Rev. Clin. Oncol.* **2017**, *14*, 717–734. [CrossRef]
40. Heindl, A.; Lan, C.; Rodrigues, D.N.; Koelble, K.; Yuan, Y. Similarity and diversity of the tumor microenvironment in multiple metastases: Critical implications for overall and progression-free survival of high-grade serous ovarian cancer. *Oncotarget* **2016**, *7*, 71123–71135. [CrossRef]
41. Jimenez-Sanchez, A.; Memon, D.; Pourpe, S.; Veeraraghavan, H.; Li, Y.; Vargas, H.A.; Gill, M.B.; Park, K.J.; Zivanovic, O.; Konner, J.; et al. Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient. *Cell* **2017**, *170*, 927–938. [CrossRef]
42. du Bois, A.; Kristensen, G.; Ray-Coquard, I.; Reuss, A.; Pignata, S.; Colombo, N.; Denison, U.; Vergote, I.; Del Campo, J.M.; Ottevanger, P.; et al. Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): A randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol.* **2016**, *17*, 78–89. [CrossRef]

43. Selle, F.; Colombo, N.; Korach, J.; Mendiola, C.; Cardona, A.; Ghazi, Y.; Oza, A.M. Safety and Efficacy of Extended Bevacizumab Therapy in Elderly (>/=70 Years) Versus Younger Patients Treated for Newly Diagnosed Ovarian Cancer in the International ROSiA Study. *Int. J. Gynecol. Cancer* **2018**, *28*, 729–737. [CrossRef] [PubMed]
44. Tewari, K.S.; Java, J.J.; Eskander, R.N.; Monk, B.J.; Burger, R.A. Early initiation of chemotherapy following complete resection of advanced ovarian cancer associated with improved survival: NRG Oncology/Gynecologic Oncology Group study. *Ann. Oncol.* **2016**, *27*, 114–121. [CrossRef] [PubMed]
45. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, D.K., AWMF). S3-Leitlinie Diagnostik, Therapie und Nachsorge maligner Ovarialtumoren, Langversion 3.0. Available online: https://www.leitlinienprogramm-onkologie.de/leitlinien/ovarialkarzinom/ (accessed on 3 June 2019).
46. Mayer, B.; Funke, I.; Johnson, J.P. High expression of a Lewis(x)-related epitope in gastric carcinomas indicates metastatic potential and poor prognosis. *Gastroenterology* **1996**, *111*, 1433–1446. [CrossRef]
47. Boussiotis, V.A. Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. *N. Engl. J. Med.* **2016**, *375*, 1767–1778. [CrossRef] [PubMed]
48. Miksch, R.C.; Hao, J.; Schoenberg, M.B.; Dotzer, K.; Schluter, F.; Weniger, M.; Yin, S.; Ormanns, S.; D'Haese, J.G.; Guba, M.O.; et al. Development of a reliable and accurate algorithm to quantify the tumor immune stroma (QTiS) across tumor types. *Oncotarget* **2017**, *8*, 114935–114944. [CrossRef]
49. Vayrynen, J.P.; Vornanen, J.O.; Sajanti, S.; Bohm, J.P.; Tuomisto, A.; Makinen, M.J. An improved image analysis method for cell counting lends credibility to the prognostic significance of T cells in colorectal cancer. *Virchows Arch.* **2012**, *460*, 455–465. [CrossRef]
50. Miksch, R.C.; Schoenberg, M.B.; Weniger, M.; Bosch, F.; Ormanns, S.; Mayer, B.; Werner, J.; Bazhin, A.V.; D'Haese, J.G. Prognostic Impact of Tumor-Infiltrating Lymphocytes and Neutrophils on Survival of Patients with Upfront Resection of Pancreatic Cancer. *Cancers* **2019**, *11*, 39. [CrossRef]
51. Schoenberg, M.B.; Hao, J.; Bucher, J.N.; Miksch, R.C.; Anger, H.J.W.; Mayer, B.; Mayerle, J.; Neumann, J.; Guba, M.O.; Werner, J.; et al. Perivascular Tumor-Infiltrating Leukocyte Scoring for Prognosis of Resected Hepatocellular Carcinoma Patients. *Cancers* **2018**, *10*, 389. [CrossRef]
52. Weiss, L.; Huemer, F.; Mlineritsch, B.; Greil, R. Immune checkpoint blockade in ovarian cancer. *Memo* **2016**, *9*, 82–84. [CrossRef]
53. Garon, E.B.; Rizvi, N.A.; Hui, R.; Leighl, N.; Balmanoukian, A.S.; Eder, J.P.; Patnaik, A.; Aggarwal, C.; Gubens, M.; Horn, L.; et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2015**, *372*, 2018–2028. [CrossRef] [PubMed]

Supplementary materials

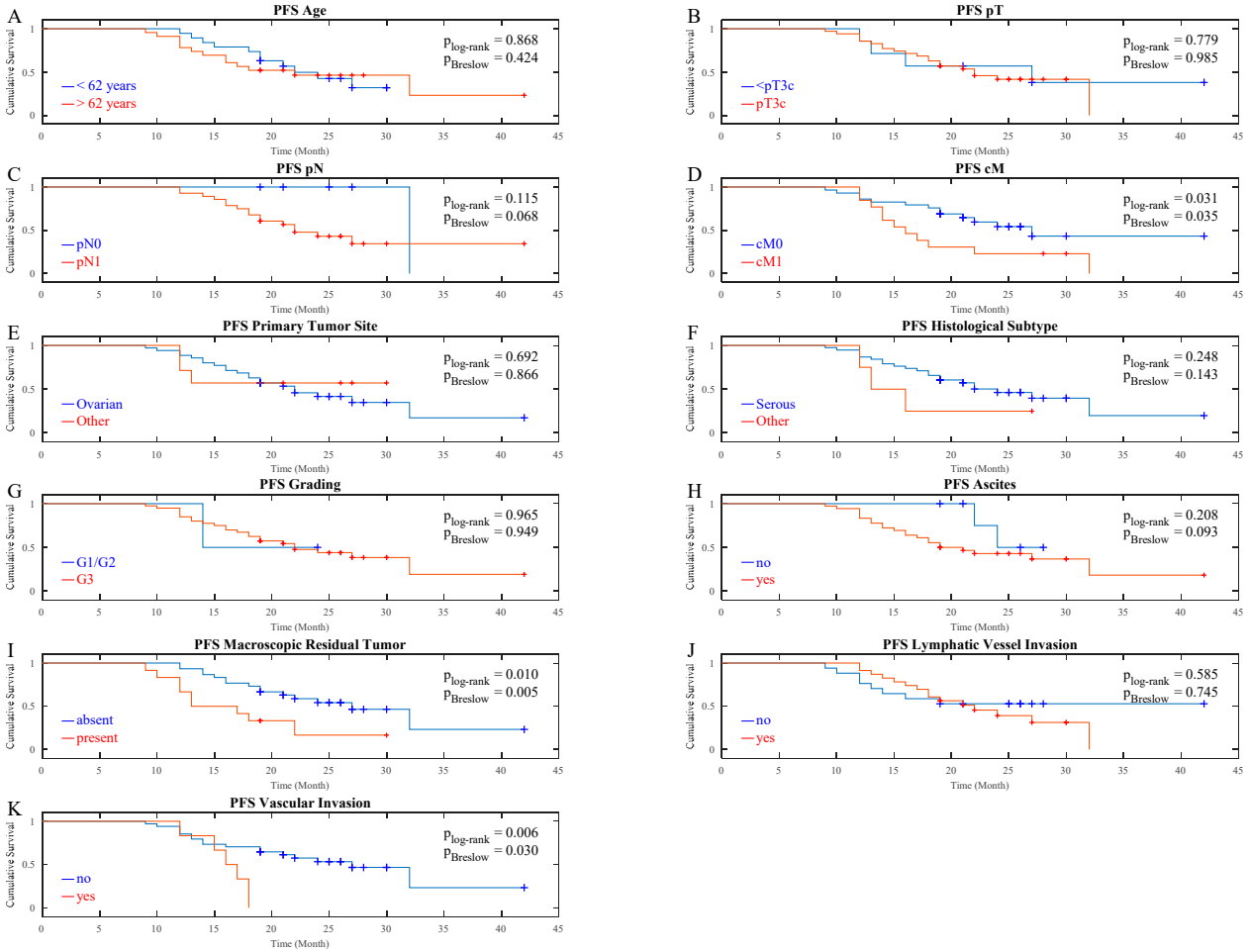

Figure S1. Kaplan-Mayer-Curves for progression free interval (PFS) of different clinicopathological parameters. (A) Age (B) pT (C) pN (D) cM (E) Primary Tumor Site (F) Histological Subtype (G) Grading (H) Ascites (I) Macroscopic Residual Tumor (J) Lymphatic Vessel Invasion (K) Vascular Invasion. p: pathological, c: clinical, T: extent of primary tumor, N: regional lymph node metastasis, M: distant metastasis. *p*-values calculated by log-rank-test and Breslow-test.

Table S1. Clinicopathological factors in relation to platinum-sensitivity.

		Platinum-Sensitivity*		
	n	**reduced**	full	$p^{\#}$
Age	42			0.191
≤ 62 years		4	15	
> 62 years		10	13	
pT	42			0.668
< pT3c		3	4	
pT3c		11	24	
pN	33			0.559
pN0		0	5	
pN1		7	21	
cM	42			**0.015**
cM0		6	23	
cM1		8	5	
Primary Tumor Site	42			0.668
Ovarian		11	24	
Other		3	4	
Histological Subtype	42			0.1
Serous		11	27	
Other		3	1	
Grading	42			1
G1/G2		1	1	
G3		13	27	
Ascites	42			0.083
No		0	6	
Yes		14	22	
Macroscopic Residual Tumor after Surgery	42			0.067
Absent		7	23	
Present		7	5	
Lymphatic Vessel Invasion	40			0.521
No		7	10	
Yes		7	16	
Vascular Invasion	40			0.159
No		10	24	
Yes		4	2	

n: number of patients, p: pathological, c: clinical, T: extent of primary tumor, N: regional lymph node metastasis, M: distant metastasis. *Platinum-sensitivity was defined as follows: reduced (relapse ≤12 months after chemotherapy) and full (relapse >12 months after chemotherapy), $^{\#}p$-value calculated by Fisher's exact two-tailed test.

Table S2. Immune cell phenotypes in primary tumor in relation to clinicopathological factors.

	Age				pT				pN				cM				Primary Tumor Site				Histological Subtype				Grading				Ascites				Macroscopic Residual Tumor				Lymphytic Vessel Invasion				Vascular Invasion			
	n	≤ 62	>62	$p^{\#}$	n	<pT3c	pT3c	$p^{\#}$	n	pN0	pN1	$p^{\#}$	n	cM0	cM1	$p^{\#}$	n	Ovarian	Other	$p^{\#}$	n	Serous	Other	$p^{\#}$	n	G1/G2	G3	$p^{\#}$	n	no	yes	$p^{\#}$	n	absent	present	$p^{\#}$	n	no	yes	$p^{\#}$	n	no	yes	$p^{\#}$
CD45 stromal	49			0.542	49			0.41	38			0.154	49			**0.042**	49			0.702	49			0.616	49			0.494	49			1	49			1	47			0.528	47			0.244
Low		7	7			1	13			0	11			13	1			12	2			12	2			1	13			2	12			10	4			5	9			13	1	
High		14	21			7	28			6	21			22	13			27	8			32	3			1	34			6	29			25	10			16	17			25	8	
CD45 intratumoral	49			0.779	49			1	38			0.663	49			1	49			1	49			1	49			1	49			**0.006**	49			1	47			0.239	47			0.486
Low		11	16			4	23			4	16			19	8			21	6			24	3			1	26			8	19			19	8			14	12			22	4	
High		10	12			4	18			2	16			16	6			18	4			20	2			1	21			0	22			16	6			7	14			16	5	
CD3 stromal	49			0.144	49			0.438	38			0.682	49			0.222	49			1	49			0.376	49			0.5	49			0.71	49			0.222	47			0.374	47			0.465
Low		15	13			6	22			3	19			22	6			22	6			24	4			2	26			4	24			22	6			14	13			23	4	
High		6	15			2	19			3	13			13	8			17	4			20	1			0	21			4	17			13	8			7	13			15	5	
CD3 intratumoral	49			0.246	49			0.715	38			0.395	49			0.53	49			0.152	49			0.056	49			1	49			1	49			0.207	47			0.076	47			0.263
Low		14	13			5	22			4	14			18	9			19	8			22	5			1	26			4	23			17	10			15	11			23	3	
High		7	15			3	19			2	18			17	5			20	2			22	0			1	21			4	18			18	4			6	15			15	6	
CD8 stromal	49			0.771	49			0.438	38			0.395	49			0.222	49			0.726	49			0.376	49			1	49			0.263	49			0.542	47			1	47			0.465
Low		13	15			6	22			2	18			22	6			23	5			24	4			1	27			3	25			21	7			12	15			23	4	
High		8	13			2	19			4	14			13	8			16	5			20	1			1	20			5	16			14	7			9	11			15	5	
CD8 intratumoral	49			**0.037**	49			0.693	38			1	49			0.527	49			0.726	49			0.143	49			1	49			1	49			0.744	47			0.562	47			0.274
Low		17	14			6	25			4	20			21	10			24	7			26	5			1	30			5	26			23	8			14	15			25	4	
High		4	14			2	16			2	12			14	4			15	3			18	0			1	17			3	15			12	6			7	11			13	5	
PD-1 stromal	49			1	49			0.202	38			0.154	49			1	49			0.702	49			1	49			1	49			1	49			0.294	47			0.528	47			**0.013**
Low		15	20			4	31			2	23			25	10			27	8			31	4			2	33			6	29			23	12			16	17			30	3	
High		6	8			4	10			4	9			10	4			12	2			13	1			0	14			2	12			12	2			5	9			8	6	
PD-1 intratumoral	49			0.76	49			0.411	38			0.392	49			0.501	49			0.464	49			1	49			1	49			1	49			1	47			0.355	47			0.121
Low		15	18			4	29			3	22			25	8			25	8			29	4			2	31			6	27			23	10			16	16			28	4	
High		6	10			4	12			3	10			10	6			14	2			15	1			0	16			2	14			12	4			5	10			10	5	
PD-L1 Positivity	49			0.376	49			1	38			0.37	49			**0.049**	49			1	49			1	49			0.145	49			1	49			1	47			0.775	47			0.278
No		10	9			3	16			1	15			17	2			15	4			17	2			2	17			3	16			14	5			9	10			17	2	
Yes		11	19			5	25			5	17			18	12			24	6			27	3			0	30			5	25			21	9			12	16			21	7	

n: number of patients, p: pathological, c: clinical, T: extent of primary tumor, N: regional lymph node metastasis, M: distant metastasis, PD-1: programmed cell-death protein 1, PD-L1: programmed cell-death ligand 1. $^{\#}$ *p*-value calculated by Fisher's exact two-tailed test.

Table S3. Comparison of the immune cell phenotypes between primary tumor and corresponding lesions.

	n PT > OM	n PT = OM	n PT < OM	$p^{\#}$	n PT > PE	n PT = PE	n PT < PE	$p^{\#}$
CD45 stromal	2	9	12	0.007	3	9	3	0.739
CD45 intratumoral	3	18	2	0.655	4	10	1	0.48
CD3 stromal	7	0	16	0.005	4	1	10	0.331
CD3 intratumoral	8	1	14	0.221	8	0	7	0.82
CD8 stromal	7	0	16	0.012	8	0	7	0.82
CD8 intratumoral	7	1	15	0.131	4	2	9	0.124
PD-1 stromal	7	1	15	0.013	8	1	6	0.95
PD-1 intratumoral	9	7	7	0.569	10	3	2	0.054
PD-L1 Expression	4	15	4	0.944	1	10	4	0.078

n: number of analyzed tumor samples, PT: Primary tumor, OM: Omental lesion, PE: Peritoneal lesion, PD-1: programmed cell-death protein 1, PD-L1: programmed cell-death ligand 1.
$^{\#}$ *p*-value calculated by Wilcoxon signed-rank test.

Table S4. Ratios of immune cell phenotypes between primary tumor and omental lesion in relation to clinicopathological factors.

	Age			pT			pN			cM			Primary Tumor Site			Histological Subtype			Grading			Ascites			Macroscopic Residual Tumor			Lymphytic Vessel Invasion			Vascular Invasion		
	n	≤ 62	>62	$p^{\#}$	n	<pT3c	pT3c	$p^{\#}$	n	pN0	pN1	$p^{\#}$	n	cM0	cM1	$p^{\#}$	n	Ovarian	Other	$p^{\#}$	n	Serous	Other	$p^{\#}$	n	G1/G2	G3	$p^{\#}$	n	no	yes	$p^{\#}$	n	absent	present	$p^{\#}$	n	no	yes	$p^{\#}$	n	no	yes	$p^{\#}$
CD45 stromal	23			0.193	23			1	19			**0.018**	23			0.590	23			0.069	23			1	23			0.478	23			0.590	23			1	22			0.395	22			0.476
OM ≤ PT		2	9			1	10			4	4			9	2			6	5			10	1			0	11			2	9			8	3			7	4			11	0	
OM > PT		6	6			1	11			0	11			11	1			11	1			10	2			2	10			1	11			8	4			4	7			9	2	
CD45 intratumoral	23			1	23			1	19			1	23			0.249	23			1	23			1	23			0.170	23			1	23			1	22			1	22			1
OM ≤ PT		7	14			2	19			4	13			19	2			15	6			18	3			1	20			3	18			14	7			10	11			19	2	
OM > PT		1	1			0	2			0	2			1	1			2	0			2	0			1	1			0	2			2	0			1	0			1	0	
CD3 stromal	23				23				19			**0.037**	23				23				23			1	23			1	23			1	23			1	22			1	22			1
OM ≤ PT		1	6			0	7			3	2			6	1			4	3			6	1			0	7			1	6			5	2			4	3			6	1	
OM > PT		7	9			2	14			1	13			14	2			13	3			14	2			2	14			2	14			11	5			7	8			14	1	
CD3 intratumoral	23			0.086	23			0.142	19			0.603	23			1	23			0.643	23			1	23			0.502	23			0.538	23			0.176	22			0.659	22			0.515
OM ≤ PT		1	8			2	7			2	5			8	1			6	3			8	1			0	9			2	7			8	1			5	3			8	0	
OM > PT		7	7			0	14			2	10			12	2			11	3			12	2			2	12			1	13			8	6			6	8			12	2	
CD8 stromal	23			1	23			1	19			0.272	23			0.526	23			0.318	23			1	23			1	23			0.209	23			0.626	22			1	22			1
OM ≤ PT		2	5			0	7			2	3			7	0			4	3			6	1			0	7			2	5			4	3			4	3			6	1	
OM > PT		6	10			2	14			2	12			13	3			13	3			14	2			2	14			1	15			12	4			7	8			14	1	
CD8 intratumoral	23			0.176	23			0.111	19			0.557	23			1	23			0.621	23			1	23			1	23			0.269	23			0.345	22			1	22			0.515
OM ≤ PT		1	7			2	6			2	4			7	1			5	3			7	1			1	7			2	6			7	1			4	4			8	0	
OM > PT		7	8			0	15			2	11			13	2			12	3			13	2			1	14			1	14			9	6			7	7			12	2	
PD-1 stromal	23			0.657	23			1	19			0.071	23			1	23			0.621	23			1	23			0.526	23			1	23			0.657	22			1	22			0.121
OM ≤ PT		2	6			1	7			3	3			7	1			5	3			7	1			0	8			1	7			5	3			4	4			6	2	
OM > PT		6	9			1	14			1	12			13	2			12	3			13	2			2	13			2	13			11	4			7	7			14	0	
PD-1 intratumoral	23			1	23			1	19			0.255	23			0.526	23			0.621	23			1	23			0.526	23			1	23			1	22			1	22			1
OM ≤ PT		6	10			2	14			4	9			13	3			11	5			14	2			1	15			2	14			11	5			8	7			13	2	
OM > PT		2	5			0	7			0	6			7	0			6	1			6	1			1	6			1	6			5	2			3	4			7	0	
PD-L1 Positivity	23			0.257	23			1	19				23			0.453	23			0.539	23			1	23			0.324	23			1	23			1	22			0.586	22			1
OM ≤ PT		8	11			2	17			3	13	0.530		17	2			13	6			16	3			1	18			3	16			13	6			10	8			16	2	
OM > PT		0	4			0	4			1	2			3	1			4	0			4	0			1	3			0	4			3	1			1	3			4	0	

n: number of patients, PT: Primary tumor, OM: Omental lesion, p: pathological, c: clinical, T: extent of primary tumor, N: regional lymph node metastasis, M: distant metastasis, PD-1: programmed cell-death protein 1, PD-L1: programmed cell-death ligand 1. $^{\#}p$-value calculated by Fisher's exact two-tailed test.

Table S5. Ratios of immune cell phenotypes between primary tumor and peritoneal lesion in relation to clinicopathological factors.

	Age				pT			pN				cM			Primary Tumor Site			Histological Subtype			Grading			Ascites			Macroscopic Residual Tumor			Lymphytic Vessel Invasion			Vascular Invasion		
	n	≤ 62	>62	$p^{\#}$	n	<pT3c	pT3c	$p^{\#}$	n	pN0	pN1	$p^{\#}$	n	cM0	cM1	$p^{\#}$	n	Ovarian	Other	$p^{\#}$	n	Serous	Other	$p^{\#}$	n	G1/G2	G3	$p^{\#}$	n	no	yes	$p^{\#}$	n	absent	present	$p^{\#}$	n	no	yes	$p^{\#}$	n	no	yes	$p^{\#}$
CD45 stromal	15			1	15			0.371	11			/	15			1	15			0.516	15			/	15			/	15			0.242	15			1	14			0.176	14			1
PE ≤ PT		6	6			1	11				10			7	5			10	2			12				12			3	9			9	3			2	9			9	2	
PE > PT		1	2			1	2				1			2	1			2	1			3				3			2	1			2	1			2	1			2	1	
CD45 intratumoral	15			0.467	15			1	11			/	15			1	15			1	15			/	15			/	15			0.333	15			1	14			1	14			1
PE ≤ PT		6	8			2	12				10			8	6			11	3			14				14			4	10			10	4			4	9			10	3	
PE > PT		1	0			0	1				1			1	0			1	0			1				1			1	0			1	0			0	1			1	0	
CD3 stromal	15			0.282	15			0.524	11			/	15			0.329	15			1	15			/	15			/	15			1	15			1	14			0.520	14			1
PE ≤ PT		1	4			0	5				4			2	3			4	1			5				5			2	3			4	1			2	2			3	1	
PE > PT		6	4			2	8				7			7	3			8	2			10				10			3	7			7	3			2	8			8	2	
CD3 intratumoral	15			0.619	15			0.467	11			/	15			0.119	15			1	15			1	15			1	15			1	15			0.569	14			0.559	14			1
PE ≤ PT		3	5			2	6				4			3	5			6	2			8		/		8		/	3	5			5	3			3	4			5	2	
PE > PT		4	3			0	7				7			6	1			6	1			7				7			2	5			6	1			1	6			6	1	
CD8 stromal	15			0.619	15			0.2	11			/	15			0.608	15			0.2	15			/	15			/	15			1	15			0.569	14			0.559	14			1
PE ≤ PT		3	5			0	8				6			4	4			5	3			8				8			3	5			5	3			3	4			5	2	
PE > PT		4	3			2	5				5			5	2			7	0			7				7			2	5			6	1			1	6			6	1	
CD8 intratumoral	15			0.119	15			0.486	11			/	15			0.622	15			0.525	15			/	15			/	15			0.329	15			1	14			0.245	14			1
PE ≤ PT		1	5			0	6				4			3	3			4	2			6				6			3	3			4	2			3	3			5	1	
PE > PT		6	3			2	7				7			6	3			8	1			9				9			2	7			7	2			1	7			6	2	
PD-1 stromal	15			0.119	15			0.486	11			/	15			0.622	15			0.229	15			/	15			/	15			0.580	15			0.604	14			0.085	14			0.538
PE ≤ PT		6	3			2	7				6			6	3			6	3			9				9			4	5			6	3			4	4			7	1	
PE > PT		1	5			0	6				5			3	3			6	0			6				6			1	5			5	1			0	6			4	2	
PD-1 intratumoral	15			0.200	15			1	11			/	15			0.486	15			0.371	15			/	15			/	15			1	15			1	14			0.505	14			1
PE ≤ PT		5	8			2	11				9			7	6			11	2			13				13			4	9			9	4			3	9			9	3	
PE > PT		2	0			0	2				2			2	0			1	1			2				2			1	1			2	0			1	1			2	0	
PD-L1 Positivity	15			1	15			1	11			/	15			0.604	15			1	15			/	15			/	15			0.231	15			1	14			0.251	14			0.505
PE ≤ PT		5	6			2	9				8			6	5			9	2			11				11			5	6			8	3			4	6			7	3	
PE > PT		2	2			0	4				3			3	1			3	1			4				4			0	4			3	1			0	4			4	0	

n: number of patients, PT: Primary tumor, PE: Peritoneal lesion, p: pathological, c: clinical, T: extent of primary tumor, N: regional lymph node metastasis, M: distant metastasis, PD-1: programmed cell-death protein 1, PD-L1: programmed cell-death ligand 1. $^{\#}p$-value calculated by Fisher's exact two-tailed test.

Table S6. Description of primary tumor site and corresponding lesions per patient.

Patient Number	Primary Tumor Site—According to Pathological Report	Location of Corresponding Lesions	
		Omentum	Peritoneum
5064	Ovary	Greater Omentum	
5085	Ovary		Peritoneum*
5188	Ovary	Greater Omentum	
4747	Ovary	Greater Omentum	Sigma
4757	Ovary		Peritoneum*
4976	Ovary		Peritoneum*
5074	Ovary	Greater Omentum	
5146	Ovary	Greater Omentum	
5196	Ovary	Greater Omentum	
5199	Ovary	Greater Omentum	
5243	Ovary	Greater Omentum	
5306	Ovary	Greater Omentum	Peritoneum*
4751	Fallopian Tube	Greater Omentum	
4754	Ovary		Peritoneum*
5072	Ovary	Greater Omentum	Sigma
5082	Fallopian Tube		Sigma
5097	Ovary	Greater Omentum	
5170	Fallopian Tube	Greater Omentum	
5216	Fallopian Tube	Greater Omentum	
5264	Ovary	Greater Omentum	Diaphragm
5266	Fallopian Tube	Greater Omentum	
5277	Peritoneum	Greater Omentum	Uterus
5281	Peritoneum	Greater Omentum	Diaphragm
4843	Ovary	Greater Omentum	
4940	Ovary		Peritoneum*
5075	Ovary		Liver Capsule
5242	Ovary	Greater Omentum	
5070	Ovary	Greater Omentum	
5088	Ovary	Greater Omentum	
5103	Ovary		Sigma
5121	Ovary	Greater Omentum	
5137	Ovary		Peritoneum*

* not further specified.

Table 7. Description of rating method.

Rating	Individual Cells	Cluster
0	none	none
1	scattered	none
2	a few	none
3	a few	small, locally restricted
4	many	small and big, locally restricted
5	many	big, confluent

biomedicines

MDPI

Article

Integrin α2β1 Represents a Prognostic and Predictive Biomarker in Primary Ovarian Cancer

Katharina Dötzer [1], Friederike Schlüter [1], Franz Edler von Koch [2], Christine E. Brambs [3], Sabine Anthuber [4], Sergio Frangini [5], Bastian Czogalla [6,7], Alexander Burges [6,7], Jens Werner [1,7], Sven Mahner [6,7] and Barbara Mayer [1,7,*]

1 Department of General, Visceral and Transplant Surgery, University Hospital, Ludwig-Maximilians-University Munich, Marchioninistraße 15, 81377 Munich, Germany; katharina.doetzer@med.uni-muenchen.de (K.D.); friederike1.schlueter@med.uni-muenchen.de (F.S.); jens.werner@med.uni-muenchen.de (J.W.)
2 Gynecology and Obstetrics Clinic, Klinikum Dritter Orden, Menzinger Straße 44, 80638 Munich, Germany; franz.koch@dritter-orden.de
3 Department of Obstetrics and Gynecology, Klinikum Rechts der Isar, Technical University Munich, Ismaninger Straße 22, 81675 Munich, Germany; christine.brambs@tum.de or christine.brambs@luks.ch
4 Department of Obstetrics and Gynecology, Starnberg Hospital, Oßwaldstraße 1, 82319 Starnberg, Germany; sabine.anthuber@klinikum-starnberg.de
5 Department of Obstetrics and Gynecology, Munich Clinic Harlaching, Sanatoriumsplatz 2, 81545 Munich, Germany; sergio.frangini@muenchen-klinik.de
6 Department of Obstetrics and Gynecology, University Hospital, Ludwig-Maximilians-University Munich, Marchioninistraße 15, 81377 Munich, Germany; bastian.czogalla@med.uni-muenchen.de (B.C.); alexander.burges@med.uni-muenchen.de (A.B.); sven.mahner@med.uni-muenchen.de (S.M.)
7 German Cancer Consortium (DKTK), Partner Site Munich, Pettenkoferstraße 8a, 80336 Munich, Germany
* Correspondence: barbara.mayer@med.uni-muenchen.de; Tel.: +49-89-4400-76438

Abstract: Currently, the same first-line chemotherapy is administered to almost all patients suffering from primary ovarian cancer. The high recurrence rate emphasizes the need for precise drug treatment in primary ovarian cancer. Being crucial in ovarian cancer progression and chemotherapeutic resistance, integrins became promising therapeutic targets. To evaluate its prognostic and predictive value, in the present study, the expression of integrin α2β1 was analyzed immunohistochemically and correlated with the survival data and other therapy-relevant biomarkers. The significant correlation of a high α2β1-expression with the estrogen receptor alpha (ERα; $p = 0.035$) and epithelial growth factor receptor (EGFR; $p = 0.027$) was observed. In addition, high α2β1-expression was significantly associated with a low number of tumor-infiltrating immune cells (CD3 intratumoral, $p = 0.017$; CD3 stromal, $p = 0.035$; PD-1 intratumoral, $p = 0.002$; PD-1 stromal, $p = 0.049$) and the lack of PD-L1 expression ($p = 0.005$). In Kaplan–Meier survival analysis, patients with a high expression of integrin α2β1 revealed a significant shorter progression-free survival (PFS, $p = 0.035$) and platinum-free interval (PFI, $p = 0.034$). In the multivariate Cox regression analysis, integrin α2β1 was confirmed as an independent prognostic factor for both PFS ($p = 0.021$) and PFI ($p = 0.020$). Dual expression of integrin α2β1 and the hepatocyte growth factor receptor (HGFR; PFS/PFI, $p = 0.004$) and CD44v6 (PFS, $p = 0.000$; PFI, $p = 0.001$; overall survival [OS], $p = 0.025$) impaired survival. Integrin α2β1 was established as a prognostic and predictive marker in primary ovarian cancer with the potential to stratify patients for chemotherapy and immunotherapy, and to design new targeted treatment strategies.

Keywords: primary ovarian cancer; integrin α2β1; prognostic factor; predictive factor; immune infiltrate; targeted therapy; personalized medicine

check for updates

Citation: Dötzer, K.; Schlüter, F.; Koch, F.E.v.; Brambs, C.E.; Anthuber, S.; Frangini, S.; Czogalla, B.; Burges, A.; Werner, J.; Mahner, S.; et al. Integrin α2β1 Represents a Prognostic and Predictive Biomarker in Primary Ovarian Cancer. *Biomedicines* **2021**, *9*, 289. https://doi.org/10.3390/biomedicines9030289

Academic Editor: Francesco Plotti

Received: 24 January 2021
Accepted: 3 March 2021
Published: 12 March 2021

1. Introduction

Several clinicopathological factors, such as advanced tumor stage and residual tumor after surgery, have been established as strong prognostic factors in primary ovarian

cancer [1]. In addition, a few tumor biological characteristics have been identified as prognostic markers. Examples are distinct gene signatures [2] or a high number of T-cells [3]. Although recently new promising candidates were detected [4], predictive markers are rare in primary ovarian cancer. Two targeted therapy approaches are recommended under current guidelines, namely vascular endothelial growth factor (VEGF) inhibition and poly (ADP-ribose) polymerase (PARP) inhibition, which are both administered in addition to the standard chemotherapy [5–7]. For VEGF inhibition, no predictive biomarker is available to select appropriate patients for anti-angiogenic therapy. Similarly, BRCA mutation or HRD status, which so far represent a prerequisite for some of the PARP inhibition treatments, need to be re-evaluated [8]. Thus, robust biomarkers for precise prognosis and treatment response are urgently required in primary ovarian cancer. This importance is emphasized by the fact that, despite standard therapy combining radical surgery and adjuvant platinum-based chemotherapy, 70–80% of the patients suffer from relapse [9].

Integrins are transmembrane cell adhesion molecules, which mediate cell–cell and cell–extracellular matrix (ECM) interaction. Currently, 18 α-subunits and 8 β-subunits are identified, forming a variety of integrin heterodimers [10]. Due to their ability of inside-out and outside-in signaling, they are known to be involved in migration, invasion, and metastasis promoting tumor progression in several cancer types [11–13]. Considering the mechanism of ovarian cancer metastasis by spreading in the peritoneal fluid and attaching to the omental and peritoneal tissue [14–16], integrins seem to be a promising therapeutic target in ovarian cancer.

While there is already some information about ovarian cancer and other β1-heterodimers, such as integrins α4β1 and α5β1 [17], less information is available about integrin α2β1. The main ligand of integrin α2β1 is collagen type I, but binding to other collagen types, laminins, and other ECM-proteins is also possible [18,19]. Expression of integrin α2β1 is not only observed on the epithelial cells, but also on the endothelial cells, platelets, white blood cells, and fibroblasts [20,21].

Previous studies indicate a role of integrin α2β1 in chemotherapy resistance [22,23], which constitutes a special interest for ovarian cancer. In the present study, the expression of integrin α2β1 in primary ovarian cancer and its prognostic and predictive role will be evaluated.

2. Materials and Methods

2.1. Study Population

Forty-eight patients diagnosed with a primary, chemonaive ovarian, fallopian tube, or peritoneal cancer from the SpheroID-Study were included. Patients suffering from another neoplasia within the last five years were excluded. Patients were recruited between September 2012 and January 2015 from five ovarian cancer centers, namely University Hospital, LMU Munich (n = 16), Klinikum Dritter Orden (n = 15), Klinikum rechts der Isar, Technical University Munich (n = 7), Munich clinic Harlaching (n = 5), and Starnberg Hospital (n = 5). Standardized surgical resection and pathological analysis was conducted by the recruiting hospital. Patient-, tumor- and treatment-related data for correlations were given in the routine reports and delivered in a pseudonymized form. Survival analysis was performed after the completion of chemotherapy. Seven patients with no chemotherapy or a reduced number of treatment cycles (≤ 2) had to be excluded. Progression-free survival (PFS) was defined as the time from surgical treatment to relapse or progression. Platinum-free interval (PFI) was defined as the time from end of the chemotherapy to relapse or progression. Overall survival (OS) was defined as the time from surgical treatment to death. Data from patients who did not die and had no relapse or progression were censored at the date of their last visit.

2.2. Immunohistochemistry

After surgical removal, tumor samples were snap frozen in liquid nitrogen. Serial cryosections (5 µm) were performed. The samples were stained immunohistochemically

using the avidin–biotin–peroxidase method [24]. Tissue sections were fixed either in acetone for 8 min or, for the antigens ERα and PgR, in formalin for 3 min and afterwards in a citrate buffer for 7 min at 90 °C. Blocking of unspecific Fc receptors was performed with 10% AB Serum (Biotest, Dreieich, Germany) in either PBS (acetone fixation) or in a TRIS–HCl buffer (formalin fixation) for 20 min. Endogenous biotin was blocked with a two-step avidin–biotin blocking kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions for 20 min. Primary antibodies were applied for one hour. Details about primary and secondary antibodies and working concentrations, including the appropriate positive and negative controls, are given in Table 1. Secondary biotinylated antibodies and peroxidase conjugated streptavidin (Dianova, Hamburg, Germany) were incubated for 30 min each.

Table 1. Biomarkers and antibodies.

Antigen	Clone	Species	Fixation	Use of Kit	wc (µg/mL)	Supplier	Cut-Off for Positivity
Primary antibodies							
Integrin α2β1	BHA2.1	m	Acetone	-	2.50	Millipore, Burlington, MA, USA	≥20%
ERα	1D5	m	Formalin	+	2.50	Dako, Santa Clara, CA, USA	≥1%
PR	PgR 636	m	Formalin	+	2.50	Dako, Santa Clara, CA, USA	≥1%
HER-2/neu	4B5	r	Acetone	-	1.50	Ventana, Roche, Basel, CH	≥10% (Intensity 2+/3+)
EGFR	H11	m	Acetone	-	2.94	Dako, Santa Clara, CA, USA	≥50%
HGFR	SP44	r	Acetone	-	2.12	Spring Bioscience, Pleasanton, CA, USA	≥50%
IGF1R	23-41	m	Acetone	+	4.00	invitrogen, Carlsbad, CA, USA	≥80%
MUC-1	Ma552	m	Acetone	-	0.50	Monosan, Uden, NL	≥70%
CD44v6	VFF-18	m	Acetone	-	1.00	affymetrix eBioscience, Santa Clara, CA, USA	≥10%
Integrin αVβ3	LM609	m	Acetone	-	5.00	Millipore, Burlington, MA, USA	≥20%
CD3	UCHT1	m	Acetone	-	1.25	BD Biosciences, Franklin Lakes, NJ, USA	
CD8	C8/144B	m	Acetone	+	3.00	Dako, Santa Clara, CA, USA	
PD-1	MIH4	m	Acetone	+	10.00	affymetrix eBioscience, Santa Clara, CA, USA	
PD-L1	MIH1	m	Acetone	+	10.00	affymetrix eBioscience, Santa Clara, CA, USA	≥1%
Positive controls							
Epithelial Antigen	Ber-EP4	m	Acetone	-	2.50	Dako, Santa Clara, CA, USA	
CD45	2B11 + PD7/26	m	Acetone	-	4.50	Dako, Santa Clara, CA, USA	
Isotype controls							
MOPC 21	MOPC 21	m		-	5.00	Sigma-Aldrich, St. Louis, MO, USA	
	MOPC 21	m		+	4.00	Sigma-Aldrich, St. Louis, MO, USA	
	MOPC 21	m		+	10.00	Sigma-Aldrich, St. Louis, MO, USA	
DA1E	DA1E	r		-	2.12	Cell Signaling, Danvers, MA, USA	
Biotin conjugated secondary antibodies							
	111-065-114	g anti r			7.00	Jackson Immunoresearch, West Grove, PA, USA	
	315-065-048	r anti m			0.75	Jackson Immunoresearch, West Grove, PA, USA	

Legend: wc: working concentration, m: mouse, r: rabbit, g: goat, all used antibodies' isotype was IgG1. ERα: estrogen receptor α, PR: progesterone receptor, Her-2/neu: human epidermal growth factor receptor 2, EGFR: epidermal growth factor receptor, HGFR: hepatocyte growth factor receptor, IGF1R: insulin-like growth factor 1, MUC-1: mucin-1, PD-1: programmed cell death protein 1, PD-L1: programmed cell death-ligand 1.

2.3. Evaluation of Biomarker Expression

Sections were evaluated semiquantitatively using a light microscope (Figure S1). The percentage of positively stained carcinoma cells was evaluated for each antigen. Tumors were defined as hormone receptor-positive if ≥1% of the cancer cells revealed a nuclear staining of ER or PR [25]. Her2/neu expression was scored according to breast cancer [26] and gastric cancer [27] guidelines. Due to the lack of further references, the other biomarkers' expression was estimated as a percentage of positive cancer cells in 10% steps. Validation was conducted by a second observer (FS). In the absence of standardized cut-offs for other biomarkers, cut-offs were evaluated according to the biphasic distribution or the group size (see Table 1). Quantitative evaluation of CD3, CD8, and PD-1, and semiquantitative evaluation of PD-L1 was performed according to Dotzer et al. [24].

2.4. Statistical Analysis

Clinicopathological factors were grouped by clinical relevance. Integrin expression was correlated with clinicopathological factors, other biomarkers' expression, and immune infiltrate using the Fisher's exact two-tailed test. Univariate analysis was performed by calculating cumulative survival probabilities with the Kaplan–Meier method and comparing them with a log-rank test. A Cox regression model was used for the multivariate analysis of survival. p-values < 0.05 were considered to be statistically significant. All statistical analyses were performed using IBM SPSS Statistics 23 (Armonk, NY, USA).

3. Results

3.1. Patient Characteristic

The clinicopathological data are shown in Table 2. Forty-eight patients were included in this study. The mean age at time of diagnosis was 62 years. Most patients suffered from high-grade, serous ovarian carcinoma in an advanced FIGO (Fédération Internationale de Gynécologie et d'Obstétrique) stage with the presence of ascites. Complete surgical resection without macroscopic residual tumor was achieved in 72.9% of all patients. In total, 83.4% of the patients received chemotherapy based on carboplatin and paclitaxel. The median OS was 42 months, the median PFS was 22 months, and the median PFI was 17 months.

Table 2. Patient characteristics.

		n or Value	%
Age	mean/median range	62/66 years 24–83 years	
FIGO Stage	I or II III IV	0 34 14	0.0% 70.8% 29.2%
pT	pT2 pT3	5 43	10.4% 89.6%
pN	pN0 pN1 Nx	6 31 11	12.5% 64.6% 22.9%
cM	cM0 cM1	34 14	70.8% 29.2%
Primary Tumor Site	Ovarian Fallopian Tube Peritoneal	39 6 3	81.3% 12.5% 6.3%
Histological Subtype	Serous Other	44 4	91.7% 8.4%
Grading	G1/G2 G3	2 46	4.2% 95.8%

Table 2. *Cont.*

		n or Value	%
Ascites	yes	40	83.3%
	no	8	16.7%
Macroscopic Residual Tumor after Surgery	None	35	72.9%
	<1 cm	6	12.5%
	>1 cm	7	14.6%
First-Line-Treatment	C	4	8.3%
	C + P	15	31.3%
	C + P + B	25	52.1%
	None	4	8.3%
Relapse after Chemotherapy	<6 months	2	4.2%
	6–12 months	12	25.0%
	>12 months	28	58.3%
	none or non-sufficient chemotherapy	6	12.5%

Legend: n: number of patients, Nx: no evaluation of lymph node status, C: carboplatin, P: paclitaxel, B: bevacizumab.

Survival data are summarized in Table 2. The presence of distant metastases (FIGO IV) was related to a shorter OS ($p = 0.015$) and tended to predict a shorter PFS ($p = 0.081$) and PFI ($p = 0.068$). Furthermore, patients with a macroscopic residual tumor after surgery showed a significant shorter OS ($p = 0.041$), PFS ($p = 0.008$) and PFI ($p = 0.01$).

3.2. Prognostic and Predictive Impact of Integrin α2β1

High integrin α2β1 expression in primary ovarian cancer was found to be associated with an unfavorable prognosis. Patients with a high expression of integrin α2β1 showed a median PFS of 16 months, which was significantly shorter compared to patients with low α2β1 expression (PFS 29 months, $p = 0.035$). In addition, high expression of integrin α2β1 predicted a shorter PFI (11 months) in contrast to patients with a low α2β1-expressing primary tumor (25 months, $p = 0.034$). Most importantly, a high expression of integrin α2β1 in primary ovarian cancer was found to be an independent prognostic factor for a shorter PFS (HR 2.46, CI 95% 1.14–5.29, $p = 0.021$) and a shorter PFI (HR 2.44, CI 95% 1.14–5.26, $p = 0.022$). No impact of the extent of α2β1 expression on OS was observed (Table 3). In addition, no significant correlation between the expression of integrin α2β1 and clinicopathological factors could be found.

Table 3. Univariate and multivariate survival analysis of clinicopathological factors and integrin α2β1.

		PFS				PFI				OS	
	n	**Log-Rank**		**MV Cox Regression**		**Log-Rank**		**MV Cox Regression**		**Log-Rank**	
		MS	***p***	**HR (CI 95%)**	***p***	**MS**	***p***	**HR (CI 95%)**	***p***	**MS**	***p***
Age ≤ 62 years	19	22	0.965			17	0.970			nr	0.193
Age > 62 years	23	22				17				42	
<pT3c	7	27	0.665			22	0.679			45	0.928
pT3c	35	22				17				42	
pN0	5	29	0.163			17	0.145			45	0.929
pN1	28	22				22				42	
cM0	29	27				22				nr	
cM1	13	16	**0.081**	2.06 (0.92–4.62)	0.081	11	**0.068**	2.10 (0.94–4.69)	**0.072**	30	**0.015**
G1/G2	2	14	0.579			8	0.610			30	0.843
G3	40	22				17				42	
Ascites absent	6	35	0.147			30	0.139			42	0.408
Ascites present	36	19				15				38	

Table 3. *Cont.*

		PFS				PFI				OS	
	n	**Log-Rank**		**MV Cox Regression**		**Log-Rank**		**MV Cox Regression**		**Log-Rank**	
		MS	***p***	**HR (CI 95%)**	***p***	**MS**	***p***	**HR (CI 95%)**	***p***	**MS**	***p***
MR Tumor absent	30	27				22				45	
MR Tumor present	12	13	**0.008**	2.19 (1.03–4.68)	**0.043**	9	**0.010**	2.10 (0.99-4.51)	0.057	26	**0.041**
Integrin α2β1 low	27	29				25				45	
Integrin α2β1 high	15	16	**0.035**	2.46 (1.14–5.29)	**0.021**	11	**0.034**	2.45 (1.14-5.26)	**0.022**	30	0.155

Legend: n: number of patients, Cox regression: multivariate Cox regression, MS: median survival (in months) in Kaplan–Meier estimator, HR: hazard ratio, CI: confidence interval, MR Tumor: macroscopic residual tumor; nr: median survival not reached.

3.3. Correlation of Integrin α2β1 with Other Biomarkers

In almost all patients (17 out of 18, 94.4%), a high expression of integrin α2β1 significantly correlated with a high expression of ERα ($p = 0.035$). Furthermore, a high expression of integrin α2β1 could be found more frequently in patients with a high expression of EGFR (7 out of 10, 70%) compared to patients with a low expression of EGFR (11 out of 38, 28.9%, $p = 0.027$, Table 4).

Table 4. Correlation between integrin α2β1 and other biomarkers.

				Integrin α2β1		
			n	**<20%**	**≥20%**	***p*** **#**
Growth Factor-Receptor	**ERα**		48			**0.035**
		<1%		10	1	
		≥1%		20	17	
	PR		48			0.127
		<1%		22	9	
		≥1%		8	9	
	Her-2/neu		48			1
		negative		22	13	
		positive		8	5	
	EGFR		48			**0.027**
		<50%		27	11	
		≥50%		3	7	
	HGFR		48			0.133
		<50%		16	5	
		≥50%		14	13	
	IGF1R		48			0.451
		<80%		4	4	
		≥80%		26	14	
Cell-Adhesion-Molecule	**MUC-1**		48			0.765
		<70%		14	7	
		≥70%		16	11	
	CD44v6		48			0.103
		<10%		24	10	
		≥10%		6	8	
	Integrin αvβ3		48			0.19
		<20%		24	11	
		≥20%		6	7	

Legend: n: number of patients, **#**: *p*-value calculated by Fisher's exact two-tailed test.

3.4. Prognostic and Predictive Impact of Integrin α2β1 Combined with Other Biomarkers

The dual expression of integrin α2β1 and various growth factor receptors revealed an impact on PFS and PFI (Table 5). Patients with a high expression of integrin α2β1 and a positive Her-2/neu status showed a shorter PFS ($p = 0.043$) and PFI ($p = 0.037$) than patients with a low expression of integrin α2β1, Her-2/neu, or both. Combined high expression of integrin α2β1 and IGF1R correlated significantly with a shorter PFS ($p = 0.045$) and PFI ($p = 0.043$). Most interestingly, a high expression of integrin α2β1 and HGFR was related to a shorter PFS ($p = 0.004$) and PFI ($p = 0.004$) and impaired prognosis in comparison to integrin α2β1 as single biomarker.

Table 5. Univariate survival analysis of dual expression of integrin α2β1 and other biomarkers.

		PFS		PFI		OS	
	n	**MS**	***p* ***	**MS**	***p* ***	**MS**	***p* ***
Integrin α2β1 high	15	16	**0.035**	11	**0.034**	30	0.155
Integrin α2β1 low	27	29		25		45	
Integrin α2β1 high/ERα high	14	16	0.078	11	0.073	30	0.287
Remaining combinations #	28	27		22		42	
Integrin α2β1 high/PR high	8	16	0.574	1119	0.578	27	0.526
Remaining combinations #	34	24		19		42	
Integrin α2β1 high/Her-2/neu +	5	21	**0.043**	15	**0.037**	36	0.698
Remaining combinations #	37	27		22		42	
Integrin α2β1 high/EGFR high	6	14	0.289	8	0.290	30	0.482
Remaining combinations #	36	22		17		42	
Integrin α2β1 high/HGFR high	11	15	**0.004**	10	**0.004**	27	0.054
Remaining combinations #	31	29		25		45	
Integrin α2β1 high/IGFR high	11	16	**0.045**	11	**0.043**	36	0.381
Remaining combinations #	31	27		22		42	
Integrin α2β1 high/MUC-1 high	9	14	0.063	9	0.055	27	0.257
Remaining combinations #	33	27		22		42	
Integrin α2β1 high/CD44v6 high	6	13	**0.000**	9	**0.001**	19	**0.025**
Remaining combinations #	36	27		22		42	
Integrin α2β1 high/Integrin αvβ3 high	5	35	0.322	30	0.320	nr	0.162
Remaining combinations #	37	22		17		42	

Legend: n: number of patients, MS: median survival (in months) in Kaplan–Meier estimator, *: *p*-value calculated by log-rank test. # The remaining combinations represent tumor samples which were integrin α2β1 high/biomarker X low, integrin α2β1 low/biomarker X high, or integrin α2β1 low/biomarker X low. nr: median survival not reached.

Likewise, a high expression of both integrin α2β1 and CD44v6 was found to be a strong factor in a poor prognosis that correlated with a shorter PFS ($p = 0.000$), PFI ($p = 0.001$) and a reduced OS ($p = 0.025$, Table 5).

3.5. Correlation of Integrin α2β1 and Immune Infiltrate

In patients with a high expression of integrin α2β1, low numbers of stromal and intratumoral CD3+ cells were found (14 out of 18, 77.8%, $p = 0.035$ and $p = 0.017$, Table 6). Furthermore, most tumors with a high expression of integrin α2β1 showed a low density of stromal (16 out of 18, 88.9%, $p = 0.049$) and intratumoral (17 out of 18, 94.4%, $p = 0.002$) PD-1+ cells. PD-L1 positivity was found more often in tumors with a low expression of integrin α2β1 (23 out of 30, 76.7%) compared to samples with a high expression (6 out of 18, 33.3%; $p = 0.005$). No correlations for CD8+ cells have been found.

Table 6. Correlations between integrin α2β1 and the immune infiltrate.

Immune Infiltrate		Integrin α2β1			
		n	<20%	≥20%	$p^{\#}$
CD3 stromal		48			**0.034**
	Low		13	14	
	High		17	4	
CD3 intratumoral		48			**0.017**
	Low		12	14	
	High		18	4	
CD8 stromal		48			0.133
	Low		14	13	
	High		16	5	
CD8 intratumoral		48			0.363
	Low		17	13	
	High		13	5	
PD-1 stromal		48			**0.049**
	Low		18	16	
	High		12	2	
PD-1 intratumoral		48			**0.002**
	Low		15	17	
	High		15	1	
PD-L1 positivity		48			**0.005**
	No		7	12	
	Yes		23	6	

Legend: n: number of patients, #: *p*-value as calculated by Fisher's exact two-tailed test.

4. Discussion

In the present study, integrin α2β1 was identified as a potential new prognostic and predictive marker in primary ovarian cancer.

A high expression of integrin α2β1 was identified as a marker for a poor prognosis with equal strength, as reported for the established clinical factors: FIGO stage and macroscopic residual tumor after surgical resection. The positive correlation between a high expression of the integrin β1 chain and short survival is documented for various tumor entities [28–30]. In particular, integrin α5β1 is already known to be an unfavorable prognostic factor for ovarian cancer [31], but also for cervical, gastric, and non-small-cell lung cancer [32–34].

Integrin α2β1 is involved in many steps of cancer progression. Binding to components of the extracellular matrix (ECM), integrin α2β1 mediates tumor cell invasion and metastasis [35–37]. This step is promoted by crosstalk with growth factor receptors [38,39]. Interestingly, in the present study, a combined expression of integrin α2β1 with ERα and EGFR was observed. Furthermore, the signaling of integrin α2β1 can induce chemoresistance. This mechanism was observed for chemotherapies containing paclitaxel [23,40], gemcitabine [41], and etoposide [42].

Early relapse and resistance to platinum-based chemotherapy are key problems in the treatment of ovarian cancer [43]. Therefore, the predictive value for the treatment response of integrin α2β1 was analyzed in the present study. Patients with a high expression of integrin α2β1 were observed to have a shorter median PFI. In particular, β1 integrins are already known to promote platinum resistance in ovarian cancer. The mechanisms of this effect are still unclear. Intracellular signaling initiated by binding to the ECM seems to be fundamental for cell adhesion-mediated drug resistance (CAM-DR) [44,45]. One of the main ECM molecules involved in this concept is collagen type I [46], which is the central binding partner of integrin α2β1 [18]. These molecular interactions suggest that the

heterodimer α2β1 contributes to CAM-DR. Therefore, targeting integrin α2β1 represents a promising strategy for overcoming platinum resistance in primary ovarian cancer.

In addition, a high expression of integrin α2β1 was observed in patients with a low density of stromal and intratumoral CD3+ as well as PD-1+ cells. Inversely, more than 75% of patients with a low expression of integrin α2β1 showed PD-L1 positivity, which represents an established predictive biomarker for immunotherapy [47]. Several integrins are related to an immunosuppressive tumor microenvironment [48,49]. For example, αv-integrins are major activators of latent TGF-β, which is involved in immunotherapy resistance [50]. The present data suggest that integrin α2β1 might play a similar role. Recently, immunotherapy became a promising approach in ovarian cancer [51,52], and phase III studies with checkpoint inhibitors in combination with platinum-based chemotherapy are already ongoing (NCT03038100, NCT03740165, NCT03737643). Low expression of integrin α2β1, therefore, could be a potential predictive marker for immunotherapy in ovarian cancer. Taken together, integrin α2β1 represents a stratification marker for patients receiving platinum-based chemotherapy and immunotherapy.

Inhibition of integrin α2β1 should be considered as a targeted therapy in ovarian cancer. Several molecules and antibodies have been developed and evaluated for integrin α2β1 inhibition in other entities.

Anti-tumoral activity was shown in prostate cancer in vivo using the monoclonal antibody GBR-500 [53]. E-7820 is a sulphonamide derivative that inhibits the expression of α2-mRNA. In Phase I studies, treatment was associated with a stable disease in a variety of malignancies [54,55]. Phase II studies are ongoing to evaluate the combination with chemotherapy in colon carcinoma (NCT01347645, NCT01133990, NCT00309179). Another β1-antibody could improve the efficiency of platinum-based chemotherapy in non-small-cell lung cancer [56]. However, despite these promising approaches, the complex biology of heterodimers with promiscuous ligands, allosteric activation, and multiple intracellular signaling pathways might hinder successful treatment strategies [13,57,58].

Furthermore, the results of this study also indicate the potential efficiency of dual inhibition. Patients with a combined high expression of integrin α2β1 and HGFR or CD44v6 showed a very short median PFS and PFI, indicating a worse prognosis and platinum resistance.

Dual targeting has become a promising strategy in ovarian cancer. Its efficiency was proven in tumor spheroid and mouse models [59,60]; thus, various phase I studies are ongoing (NCT03895788, NCT03695380, NCT04315233). In future studies, dual inhibition including integrin α2β1-antagonists should be considered for patients with an appropriate biomarker profile.

The main limitation of this study is the small cohort, though it is representative and comparable to cohorts of other clinical trials. The promising role of integrin α2β1 as a new prognostic and predictive biomarker in primary ovarian cancer needs to be confirmed by an enlarged study.

5. Conclusions

In the present study, integrin α2β1 was identified as a prognostic and predictive marker in primary ovarian cancer. High expression of integrin α2β1 correlated with a short PFS. Prognosis was even worse in integrin α2β1-positive tumors co-expressing HGFR or CD44v6. This finding might lead to new biomarker-directed treatment strategies in primary ovarian cancer. In addition, the high expression of integrin α2β1 correlated with a short PFI, supporting the hypothesis that integrins mediate platinum resistance. Thus, a high expression of integrin α2β1 might represent a stratification marker for personalized treatment.

Supplementary Materials: The following are available online at https://www.mdpi.com/2227-9059/9/3/289/s1, Figure S1: Immunohistochemical stainings.

Author Contributions: Conceptualization, B.M.; formal analysis, K.D. and B.M.; funding acquisition, B.M.; investigation, K.D.; methodology, K.D., F.S. and B.M.; project administration, B.M.; resources, F.E.v.K., C.E.B., S.A., S.F., B.C., A.B. and S.M.; validation, F.S., B.C. and B.M.; visualization, K.D.; writing—original draft, K.D. and B.M.; writing—review and editing, F.S., C.E.B., S.F., J.W. and S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the German Federal Ministry of Education and Research (Leading Edge Cluster m4) to B.M., Grant FKZ 16EX1021N.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the institutional review board at 07th August 2012 of the Ludwig-Maximilians-Universität, Munich, Germany (No.278/04).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to protection of detailed patient-related data.

Acknowledgments: The authors appreciate Maja Schorkowitz for acquisition of follow-up data and Anton Stolp, Frank Arnold and Michael Pohr for technical support.

Conflicts of Interest: All but one author declare no conflict of interest. Sven Mahner: Research support, advisory board, honoraria and travel expenses from AbbVie, AstraZeneca, Clovis, Eisai, GlaxoSmithKline, Medac, MSD, Novartis, Olympus, PharmaMar, Roche, Sensor Kinesis, Teva and Tesaro. This support was not related to work in the present study. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. du Bois, A.; Reuss, A.; Pujade-Lauraine, E.; Harter, P.; Ray-Coquard, I.; Pfisterer, J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: A combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: By the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). *Cancer* **2009**, *115*, 1234–1244. [CrossRef]
2. Waldron, L.; Haibe-Kains, B.; Culhane, A.C.; Riester, M.; Ding, J.; Wang, X.V.; Ahmadifar, M.; Tyekucheva, S.; Bernau, C.; Risch, T.; et al. Comparative meta-analysis of prognostic gene signatures for late-stage ovarian cancer. *J. Natl. Cancer Inst.* **2014**, *106*. [CrossRef]
3. Li, J.; Wang, J.; Chen, R.; Bai, Y.; Lu, X. The prognostic value of tumor-infiltrating T lymphocytes in ovarian cancer. *Oncotarget* **2017**, *8*, 15621–15631. [CrossRef] [PubMed]
4. Plotti, F.; Terranova, C.; Guzzo, F.; De Cicco Nardone, C.; Luvero, D.; Bartolone, M.; Dionisi, C.; Benvenuto, D.; Fabris, S.; Ciccozzi, M.; et al. Role of BRCA Mutation and HE4 in Predicting Chemotherapy Response in Ovarian Cancer: A Retrospective Pilot Study. *Biomedicines* **2021**, *9*, 55. [CrossRef]
5. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, D.K., AWMF). S3-Leitlinie Diagnostik, Therapie und Nachsorge Maligner Ovarialtumoren, Langversion 4.0. Available online: https://www.leitlinienprogramm-onkologie.de/leitlinien/ovarialkarzinom/ (accessed on 24 January 2021).
6. National Institute for Health and Care Excellence. Olaparib for Maintenance Treatment of BRCA Mutation-Positive Advanced Ovarian, Fallopian Tube or Peritoneal Cancer after Response to First-Line Platinum-Based Chemotherapy. Available online: https://www.nice.org.uk/guidance/ta598 (accessed on 24 January 2021).
7. Armstrong, D.K.; Alvarez, R.D.; Bakkum-Gamez, J.N.; Barroilhet, L.; Behbakht, K.; Berchuck, A.; Berek, J.S.; Chen, L.M.; Cristea, M.; DeRosa, M.; et al. NCCN Guidelines Insights: Ovarian Cancer, Version 1.2019. *J. Natl. Compr. Cancer Netw.* **2019**, *17*, 896–909. [CrossRef] [PubMed]
8. Colombo, N.; Sessa, C.; du Bois, A.; Ledermann, J.; McCluggage, W.G.; McNeish, I.; Morice, P.; Pignata, S.; Ray-Coquard, I.; Vergote, I.; et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: Pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. *Ann. Oncol.* **2019**, *30*, 672–705. [CrossRef]
9. Pignata, S.; Cecere, S.C.; Du Bois, A.; Harter, P.; Heitz, F. Treatment of recurrent ovarian cancer. *Ann. Oncol.* **2017**, *28*, viii51–viii56. [CrossRef] [PubMed]
10. Hynes, R.O. Integrins: Bidirectional, allosteric signaling machines. *Cell* **2002**, *110*, 673–687. [CrossRef]
11. Bianconi, D.; Unseld, M.; Prager, G.W. Integrins in the Spotlight of Cancer. *Int. J. Mol. Sci.* **2016**, *17*, 2037. [CrossRef] [PubMed]
12. Ganguly, K.K.; Pal, S.; Moulik, S.; Chatterjee, A. Integrins and metastasis. *Cell Adh. Migr.* **2013**, *7*, 251–261. [CrossRef] [PubMed]
13. Alday-Parejo, B.; Stupp, R.; Rüegg, C. Are Integrins Still Practicable Targets for Anti-Cancer Therapy? *Cancers* **2019**, *11*, 978. [CrossRef] [PubMed]
14. Lengyel, E. Ovarian cancer development and metastasis. *Am. J. Pathol.* **2010**, *177*, 1053–1064. [CrossRef]

15. Aziz, M.; Agarwal, K.; Dasari, S.; Mitra, A.A.K. Productive Cross-Talk with the Microenvironment: A Critical Step in Ovarian Cancer Metastasis. *Cancers* **2019**, *11*, 1608. [CrossRef]
16. Ghoneum, A.; Afify, H.; Salih, Z.; Kelly, M.; Said, N. Role of tumor microenvironment in ovarian cancer pathobiology. *Oncotarget* **2018**, *9*, 22832–22849. [CrossRef] [PubMed]
17. Kobayashi, M.; Sawada, K.; Kimura, T. Potential of Integrin Inhibitors for Treating Ovarian Cancer: A Literature Review. *Cancers* **2017**, *9*, 83. [CrossRef]
18. Heino, J. The collagen receptor integrins have distinct ligand recognition and signaling functions. *Matrix Biol.* **2000**, *19*, 319–323. [CrossRef]
19. Humphries, J.D.; Byron, A.; Humphries, M.J. Integrin ligands at a glance. *J. Cell Sci.* **2006**, *119*, 3901–3903. [CrossRef] [PubMed]
20. Van de Walle, G.R.; Vanhoorelbeke, K.; Majer, Z.; Illyés, E.; Baert, J.; Pareyn, I.; Deckmyn, H. Two functional active conformations of the integrin {alpha}2{beta}1, depending on activation condition and cell type. *J. Biol. Chem.* **2005**, *280*, 36873–36882. [CrossRef]
21. Zutter, M.M.; Santoro, S.A. Widespread histologic distribution of the alpha 2 beta 1 integrin cell-surface collagen receptor. *Am. J. Pathol.* **1990**, *137*, 113–120.
22. Naci, D.; Vuori, K.; Aoudjit, F. Alpha2beta1 integrin in cancer development and chemoresistance. *Semin. Cancer Biol.* **2015**, *35*, 145–153. [CrossRef]
23. Zheng, W.; Ge, D.; Meng, G. Reversing microtubule-directed chemotherapeutic drug resistance by co-delivering alpha2beta1 inhibitor and paclitaxel with nanoparticles in ovarian cancer. *Cell Biol. Int.* **2020**, *44*, 610–620. [CrossRef] [PubMed]
24. Dotzer, K.; Schluter, F.; Schoenberg, M.B.; Bazhin, A.V.; von Koch, F.E.; Schnelzer, A.; Anthuber, S.; Grab, D.; Czogalla, B.; Burges, A.; et al. Immune Heterogeneity Between Primary Tumors and Corresponding Metastatic Lesions and Response to Platinum Therapy in Primary Ovarian Cancer. *Cancers* **2019**, *11*, 1250. [CrossRef] [PubMed]
25. Sieh, W.; Kobel, M.; Longacre, T.A.; Bowtell, D.D.; deFazio, A.; Goodman, M.T.; Hogdall, E.; Deen, S.; Wentzensen, N.; Moysich, K.B.; et al. Hormone-receptor expression and ovarian cancer survival: An Ovarian Tumor Tissue Analysis consortium study. *Lancet Oncol.* **2013**, *14*, 853–862. [CrossRef]
26. Wolff, A.C.; Hammond, M.E.H.; Allison, K.H.; Harvey, B.E.; Mangu, P.B.; Bartlett, J.M.S.; Bilous, M.; Ellis, I.O.; Fitzgibbons, P.; Hanna, W.; et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J. Clin. Oncol.* **2018**, *36*, 2105–2122. [CrossRef] [PubMed]
27. Ruschoff, J.; Dietel, M.; Baretton, G.; Arbogast, S.; Walch, A.; Monges, G.; Chenard, M.P.; Penault-Llorca, F.; Nagelmeier, I.; Schlake, W.; et al. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. *Virchows Arch.* **2010**, *457*, 299–307. [CrossRef]
28. Yao, E.S.; Zhang, H.; Chen, Y.Y.; Lee, B.; Chew, K.; Moore, D.; Park, C. Increased beta1 integrin is associated with decreased survival in invasive breast cancer. *Cancer Res.* **2007**, *67*, 659–664. [CrossRef]
29. Liu, Q.Z.; Gao, X.H.; Chang, W.J.; Gong, H.F.; Fu, C.G.; Zhang, W.; Cao, G.W. Expression of ITGB1 predicts prognosis in colorectal cancer: A large prospective study based on tissue microarray. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 12802–12810.
30. Sun, Q.; Zhou, C.; Ma, R.; Guo, Q.; Huang, H.; Hao, J.; Liu, H.; Shi, R.; Liu, B. Prognostic value of increased integrin-beta 1 expression in solid cancers: A meta-analysis. *OncoTargets Ther.* **2018**, *11*, 1787–1799. [CrossRef] [PubMed]
31. Sawada, K.; Mitra, A.K.; Radjabi, A.R.; Bhaskar, V.; Kistner, E.O.; Tretiakova, M.; Jagadeeswaran, S.; Montag, A.; Becker, A.; Kenny, H.A.; et al. Loss of E-cadherin promotes ovarian cancer metastasis via alpha 5-integrin, which is a therapeutic target. *Cancer Res.* **2008**, *68*, 2329–2339. [CrossRef]
32. Adachi, M.; Taki, T.; Higashiyama, M.; Kohno, N.; Inufusa, H.; Miyake, M. Significance of integrin alpha5 gene expression as a prognostic factor in node-negative non-small cell lung cancer. *Clin. Cancer Res.* **2000**, *6*, 96–101.
33. Ren, J.; Xu, S.; Guo, D.; Zhang, J.; Liu, S. Increased expression of α5β1-integrin is a prognostic marker for patients with gastric cancer. *Clin. Transl. Oncol.* **2014**, *16*, 668–674. [CrossRef] [PubMed]
34. Wang, H.Y.; Chen, Z.; Wang, Z.H.; Wang, H.; Huang, L.M. Prognostic significance of α5β1-integrin expression in cervical cancer. *Asian Pac. J. Cancer Prev.* **2013**, *14*, 3891–3895. [CrossRef]
35. Shield, K.; Riley, C.; Quinn, M.A.; Rice, G.E.; Ackland, M.L.; Ahmed, N. Alpha2beta1 integrin affects metastatic potential of ovarian carcinoma spheroids by supporting disaggregation and proteolysis. *J. Carcinog.* **2007**, *6*, 11. [CrossRef]
36. Burnier, J.V.; Wang, N.; Michel, R.P.; Hassanain, M.; Li, S.; Lu, Y.; Metrakos, P.; Antecka, E.; Burnier, M.N.; Ponton, A.; et al. Type IV collagen-initiated signals provide survival and growth cues required for liver metastasis. *Oncogene* **2011**, *30*, 3766–3783. [CrossRef]
37. Bartolomé, R.A.; Barderas, R.; Torres, S.; Fernandez-Aceñero, M.J.; Mendes, M.; García-Foncillas, J.; Lopez-Lucendo, M.; Casal, J.I. Cadherin-17 interacts with α2β1 integrin to regulate cell proliferation and adhesion in colorectal cancer cells causing liver metastasis. *Oncogene* **2014**, *33*, 1658–1669. [CrossRef] [PubMed]
38. Eliceiri, B.P. Integrin and growth factor receptor crosstalk. *Circ. Res.* **2001**, *89*, 1104–1110. [CrossRef]
39. Hamidi, H.; Ivaska, J. Every step of the way: Integrins in cancer progression and metastasis. *Nat. Rev. Cancer* **2018**, *18*, 533–548. [CrossRef] [PubMed]
40. Aoudjit, F.; Vuori, K. Integrin signaling inhibits paclitaxel-induced apoptosis in breast cancer cells. *Oncogene* **2001**, *20*, 4995–5004. [CrossRef] [PubMed]

41. Duxbury, M.S.; Ito, H.; Benoit, E.; Waseem, T.; Ashley, S.W.; Whang, E.E. RNA interference demonstrates a novel role for integrin-linked kinase as a determinant of pancreatic adenocarcinoma cell gemcitabine chemoresistance. *Clin. Cancer Res.* **2005**, *11*, 3433–3438. [CrossRef] [PubMed]
42. Sethi, T.; Rintoul, R.C.; Moore, S.M.; MacKinnon, A.C.; Salter, D.; Choo, C.; Chilvers, E.R.; Dransfield, I.; Donnelly, S.C.; Strieter, R.; et al. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: A mechanism for small cell lung cancer growth and drug resistance in vivo. *Nat. Med.* **1999**, *5*, 662–668. [CrossRef]
43. Wilson, M.K.; Pujade-Lauraine, E.; Aoki, D.; Mirza, M.R.; Lorusso, D.; Oza, A.M.; du Bois, A.; Vergote, I.; Reuss, A.; Bacon, M.; et al. Fifth Ovarian Cancer Consensus Conference of the Gynecologic Cancer InterGroup: Recurrent disease. *Ann. Oncol.* **2017**, *28*, 727–732. [CrossRef] [PubMed]
44. Scalici, J.M.; Harrer, C.; Allen, A.; Jazaeri, A.; Atkins, K.A.; McLachlan, K.R.; Slack-Davis, J.K. Inhibition of alpha4beta1 integrin increases ovarian cancer response to carboplatin. *Gynecol. Oncol.* **2014**, *132*, 455–461. [CrossRef]
45. Wantoch von Rekowski, K.; König, P.; Henze, S.; Schlesinger, M.; Zawierucha, P.; Januchowski, R.; Bendas, G. The Impact of Integrin-Mediated Matrix Adhesion on Cisplatin Resistance of W1 Ovarian Cancer Cells. *Biomolecules* **2019**, *9*, 788. [CrossRef] [PubMed]
46. Baltes, F.; Pfeifer, V.; Silbermann, K.; Caspers, J.; Wantoch von Rekowski, K.; Schlesinger, M.; Bendas, G. β(1)-Integrin binding to collagen type 1 transmits breast cancer cells into chemoresistance by activating ABC efflux transporters. *Biochim. Biophys. Acta Mol. Cell Res.* **2020**, *1867*, 118663. [CrossRef] [PubMed]
47. Gibney, G.T.; Weiner, L.M.; Atkins, M.B. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol.* **2016**, *17*, e542–e551. [CrossRef]
48. Schmid, M.C.; Avraamides, C.J.; Dippold, H.C.; Franco, I.; Foubert, P.; Ellies, L.G.; Acevedo, L.M.; Manglicmot, J.R.; Song, X.; Wrasidlo, W.; et al. Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3kγ, a single convergent point promoting tumor inflammation and progression. *Cancer Cell* **2011**, *19*, 715–727. [CrossRef]
49. Wu, A.; Zhang, S.; Liu, J.; Huang, Y.; Deng, W.; Shu, G.; Yin, G. Integrated Analysis of Prognostic and Immune Associated Integrin Family in Ovarian Cancer. *Front. Genet.* **2020**, *11*, 705. [CrossRef]
50. Brown, N.F.; Marshall, J.F. Integrin-Mediated TGFbeta Activation Modulates the Tumour Microenvironment. *Cancers* **2019**, *11*, 1221. [CrossRef]
51. Kandalaft, L.E.; Odunsi, K.; Coukos, G. Immunotherapy in Ovarian Cancer: Are We There Yet? *J. Clin. Oncol.* **2019**, *37*, 2460–2471. [CrossRef]
52. Wang, W.; Liu, J.R.; Zou, W. Immunotherapy in Ovarian Cancer. *Surg. Oncol. Clin. N. Am.* **2019**, *28*, 447–464. [CrossRef]
53. Attinger, A.; Hou, S.; Pesenti, E.A.; Mancini, L.; Mottl, H. Abstract LB-294: GBR 500, a monoclonal VLA-2 antibody inhibits tumor and metastasis growth but not extravasation in a prostate cancer animal model. In Proceedings of the AACR 102nd Annual Meeting 2011, Orlando, FL, USA, 2–6 April 2011.
54. Milojkovic Kerklaan, B.; Slater, S.; Flynn, M.; Greystoke, A.; Witteveen, P.O.; Megui-Roelvink, M.; de Vos, F.; Dean, E.; Reyderman, L.; Ottesen, L.; et al. A phase I, dose escalation, pharmacodynamic, pharmacokinetic, and food-effect study of alpha2 integrin inhibitor E7820 in patients with advanced solid tumors. *Investig. New Drugs* **2016**, *34*, 329–337. [CrossRef] [PubMed]
55. Mita, M.; Kelly, K.R.; Mita, A.; Ricart, A.D.; Romero, O.; Tolcher, A.; Hook, L.; Okereke, C.; Krivelevich, I.; Rossignol, D.P.; et al. Phase I study of E7820, an oral inhibitor of integrin alpha-2 expression with antiangiogenic properties, in patients with advanced malignancies. *Clin. Cancer Res.* **2011**, *17*, 193–200. [CrossRef] [PubMed]
56. Kim, M.Y.; Cho, W.D.; Hong, K.P.; Choi da, B.; Hong, J.W.; Kim, S.; Moon, Y.R.; Son, S.M.; Lee, O.J.; Lee, H.C.; et al. Novel monoclonal antibody against beta 1 integrin enhances cisplatin efficacy in human lung adenocarcinoma cells. *J. Biomed. Res.* **2016**, *30*, 217–224. [CrossRef] [PubMed]
57. Raab-Westphal, S.; Marshall, J.F.; Goodman, S.L. Integrins as Therapeutic Targets: Successes and Cancers. *Cancers* **2017**, *9*, 110. [CrossRef]
58. Millard, M.; Odde, S.; Neamati, N. Integrin targeted therapeutics. *Theranostics* **2011**, *1*, 154–188. [CrossRef]
59. Taylan, E.; Zayou, F.; Murali, R.; Karlan, B.Y.; Pandol, S.J.; Edderkaoui, M.; Orsulic, S. Dual targeting of GSK3B and HDACs reduces tumor growth and improves survival in an ovarian cancer mouse model. *Gynecol. Oncol.* **2020**, *159*, 277–284. [CrossRef] [PubMed]
60. Camblin, A.J.; Tan, G.; Curley, M.D.; Yannatos, I.; Iadevaia, S.; Rimkunas, V.; Mino-Kenudson, M.; Bloom, T.; Schoeberl, B.; Drummond, D.C.; et al. Dual targeting of IGF-1R and ErbB3 as a potential therapeutic regimen for ovarian cancer. *Sci. Rep.* **2019**, *9*, 16832. [CrossRef]

4. References

1. Ferlay, J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today. Availabe online: https://gco.iarc.fr/today (accessed on 23 February 2022).
2. Robert Koch-Institut und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. Krebs in Deutschland für 2017/2018. Availabe online: http://www.krebsdaten.de/Krebs/DE/Content/Publikationen/Krebs_in_Deutschland/krebs_in_deutschland_node.html (accessed on 23 February 2022).
3. Menon, U.; Karpinskyj, C.; Gentry-Maharaj, A. Ovarian Cancer Prevention and Screening. *Obstet Gynecol* **2018**, *131*, 909-927, doi:10.1097/aog.0000000000002580.
4. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, D.K., AWMF). S3-Leitlinie Diagnostik, Therapie und Nachsorge maligner Ovarialtumoren, Langversion 5.0. Availabe online: https://www.leitlinienprogramm-onkologie.de/leitlinien/ovarialkarzinom/ (accessed on 23 February 2022).
5. Bankhead, C.R.; Kehoe, S.T.; Austoker, J. Symptoms associated with diagnosis of ovarian cancer: a systematic review. *Bjog* **2005**, *112*, 857-865, doi:10.1111/j.1471-0528.2005.00572.x.
6. Berek, J.S.; Kehoe, S.T.; Kumar, L.; Friedlander, M. Cancer of the ovary, fallopian tube, and peritoneum. *Int J Gynaecol Obstet* **2018**, *143 Suppl 2*, 59-78, doi:10.1002/ijgo.12614.
7. du Bois, A.; Reuss, A.; Pujade-Lauraine, E.; Harter, P.; Ray-Coquard, I.; Pfisterer, J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). *Cancer* **2009**, *115*, 1234-1244, doi:10.1002/cncr.24149.
8. du Bois, A.; Kristensen, G.; Ray-Coquard, I.; Reuss, A.; Pignata, S.; Colombo, N.; Denison, U.; Vergote, I.; Del Campo, J.M.; Ottevanger, P.; Heubner, M.; Minarik, T.; Sevin, E.; de Gregorio, N.; Bidzinski, M.; Pfisterer, J.; Malander, S.; Hilpert, F.; Mirza, M.R.; Scambia, G.; Meier, W.; Nicoletto, M.O.; Bjorge, L.; Lortholary, A.; Sailer, M.O.; Merger, M.; Harter, P. Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol* **2016**, *17*, 78-89, doi:10.1016/s1470-2045(15)00366-6.
9. Oza, A.M.; Cook, A.D.; Pfisterer, J.; Embleton, A.; Ledermann, J.A.; Pujade-Lauraine, E.; Kristensen, G.; Carey, M.S.; Beale, P.; Cervantes, A.; Park-Simon, T.W.; Rustin, G.; Joly, F.; Mirza, M.R.; Plante, M.; Quinn, M.; Poveda, A.; Jayson, G.C.; Stark, D.; Swart, A.M.; Farrelly, L.; Kaplan, R.; Parmar, M.K.; Perren, T.J. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. *Lancet Oncol* **2015**, *16*, 928-936, doi:10.1016/s1470-2045(15)00086-8.
10. Ozols, R.F.; Bundy, B.N.; Greer, B.E.; Fowler, J.M.; Clarke-Pearson, D.; Burger, R.A.; Mannel, R.S.; DeGeest, K.; Hartenbach, E.M.; Baergen, R. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol* **2003**, *21*, 3194-3200, doi:10.1200/jco.2003.02.153.

11. Pignata, S.; Scambia, G.; Katsaros, D.; Gallo, C.; Pujade-Lauraine, E.; De Placido, S.; Bologna, A.; Weber, B.; Raspagliesi, F.; Panici, P.B.; Cormio, G.; Sorio, R.; Cavazzini, M.G.; Ferrandina, G.; Breda, E.; Murgia, V.; Sacco, C.; Cinieri, S.; Salutari, V.; Ricci, C.; Pisano, C.; Greggi, S.; Lauria, R.; Lorusso, D.; Marchetti, C.; Selvaggi, L.; Signoriello, S.; Piccirillo, M.C.; Di Maio, M.; Perrone, F. Carboplatin plus paclitaxel once a week versus every 3 weeks in patients with advanced ovarian cancer (MITO-7): a randomised, multicentre, open-label, phase 3 trial. *Lancet Oncol* **2014**, *15*, 396-405, doi:10.1016/s1470-2045(14)70049-x.

12. Lheureux, S.; Braunstein, M.; Oza, A.M. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. *CA Cancer J Clin* **2019**, *69*, 280-304, doi:10.3322/caac.21559.

13. Garrido, M.P.; Fredes, A.N.; Lobos-González, L.; Valenzuela-Valderrama, M.; Vera, D.B.; Romero, C. Current Treatments and New Possible Complementary Therapies for Epithelial Ovarian Cancer. *Biomedicines* **2021**, *10*, doi:10.3390/biomedicines10010077.

14. Kurnit, K.C.; Fleming, G.F.; Lengyel, E. Updates and New Options in Advanced Epithelial Ovarian Cancer Treatment. *Obstet Gynecol* **2021**, *137*, 108-121, doi:10.1097/aog.0000000000004173.

15. Colombo, N.; Sessa, C.; du Bois, A.; Ledermann, J.; McCluggage, W.G.; McNeish, I.; Morice, P.; Pignata, S.; Ray-Coquard, I.; Vergote, I.; Baert, T.; Belaroussi, I.; Dashora, A.; Olbrecht, S.; Planchamp, F.; Querleu, D. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. *Ann Oncol* **2019**, *30*, 672-705, doi:10.1093/annonc/mdz062.

16. Pignata, S.; S, C.C.; Du Bois, A.; Harter, P.; Heitz, F. Treatment of recurrent ovarian cancer. *Ann Oncol* **2017**, *28*, viii51-viii56, doi:10.1093/annonc/mdx441.

17. Piccart, M.J.; Bertelsen, K.; James, K.; Cassidy, J.; Mangioni, C.; Simonsen, E.; Stuart, G.; Kaye, S.; Vergote, I.; Blom, R.; Grimshaw, R.; Atkinson, R.J.; Swenerton, K.D.; Trope, C.; Nardi, M.; Kaern, J.; Tumolo, S.; Timmers, P.; Roy, J.A.; Lhoas, F.; Lindvall, B.; Bacon, M.; Birt, A.; Andersen, J.E.; Zee, B.; Paul, J.; Baron, B.; Pecorelli, S. Randomized intergroup trial of cisplatin-paclitaxel versus cisplatin-cyclophosphamide in women with advanced epithelial ovarian cancer: three-year results. *J Natl Cancer Inst* **2000**, *92*, 699-708.

18. Roett, M.A.; Evans, P. Ovarian cancer: an overview. *Am Fam Physician* **2009**, *80*, 609-616.

19. Winter, W.E., 3rd; Maxwell, G.L.; Tian, C.; Carlson, J.W.; Ozols, R.F.; Rose, P.G.; Markman, M.; Armstrong, D.K.; Muggia, F.; McGuire, W.P. Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. *J Clin Oncol* **2007**, *25*, 3621-3627, doi:10.1200/jco.2006.10.2517.

20. Ataseven, B.; Grimm, C.; Harter, P.; Heitz, F.; Traut, A.; Prader, S.; du Bois, A. Prognostic impact of debulking surgery and residual tumor in patients with epithelial ovarian cancer FIGO stage IV. *Gynecol Oncol* **2016**, *140*, 215-220, doi:10.1016/j.ygyno.2015.12.007.

21. Kommoss, S.; du Bois, A.; Ridder, R.; Trunk, M.J.; Schmidt, D.; Pfisterer, J.; Kommoss, F. Independent prognostic significance of cell cycle regulator proteins p16(INK4a) and pRb in advanced-stage ovarian carcinoma including optimally debulked patients: a translational research subprotocol of a randomised study of the Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group. *Br J Cancer* **2007**, *96*, 306-313, doi:10.1038/sj.bjc.6603531.

22. Waldron, L.; Haibe-Kains, B.; Culhane, A.C.; Riester, M.; Ding, J.; Wang, X.V.; Ahmadifar, M.; Tyekucheva, S.; Bernau, C.; Risch, T.; Ganzfried, B.F.; Huttenhower, C.; Birrer, M.; Parmigiani, G. Comparative meta-analysis of prognostic gene signatures for late-stage ovarian cancer. *J Natl Cancer Inst* **2014**, *106*, doi:10.1093/jnci/dju049.

23. Hwang, W.T.; Adams, S.F.; Tahirovic, E.; Hagemann, I.S.; Coukos, G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. *Gynecol Oncol* **2012**, *124*, 192-198, doi:10.1016/j.ygyno.2011.09.039.

24. Li, J.; Wang, J.; Chen, R.; Bai, Y.; Lu, X. The prognostic value of tumor-infiltrating T lymphocytes in ovarian cancer. *Oncotarget* **2017**, *8*, 15621-15631, doi:10.18632/oncotarget.14919.

25. Lengyel, E. Ovarian cancer development and metastasis. *Am J Pathol* **2010**, *177*, 1053-1064, doi:10.2353/ajpath.2010.100105.

26. Ghoneum, A.; Afify, H.; Salih, Z.; Kelly, M.; Said, N. Role of tumor microenvironment in ovarian cancer pathobiology. *Oncotarget* **2018**, *9*, 22832-22849, doi:10.18632/oncotarget.25126.

27. Worzfeld, T.; Pogge von Strandmann, E.; Huber, M.; Adhikary, T.; Wagner, U.; Reinartz, S.; Muller, R. The Unique Molecular and Cellular Microenvironment of Ovarian Cancer. *Front Oncol* **2017**, *7*, 24, doi:10.3389/fonc.2017.00024.

28. Dasari, S.; Fang, Y.; Mitra, A.K. Cancer Associated Fibroblasts: Naughty Neighbors That Drive Ovarian Cancer Progression. *Cancers (Basel)* **2018**, *10*, doi:10.3390/cancers10110406.

29. Cai, D.L.; Jin, L.P. Immune Cell Population in Ovarian Tumor Microenvironment. *J Cancer* **2017**, *8*, 2915-2923, doi:10.7150/jca.20314.

30. Boussiotis, V.A. Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. *N Engl J Med* **2016**, *375*, 1767-1778, doi:10.1056/NEJMra1514296.

31. Ostrand-Rosenberg, S.; Horn, L.A.; Haile, S.T. The programmed death-1 immune-suppressive pathway: barrier to antitumor immunity. *J Immunol* **2014**, *193*, 3835-3841, doi:10.4049/jimmunol.1401572.

32. Schildberg, F.A.; Klein, S.R.; Freeman, G.J.; Sharpe, A.H. Coinhibitory Pathways in the B7-CD28 Ligand-Receptor Family. *Immunity* **2016**, *44*, 955-972, doi:10.1016/j.immuni.2016.05.002.

33. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, D.K., AWMF). S3-LeitliniePrävention, Diagnostik, Therapie und Nachsorge des Lungenkarzinoms, Langversion 1.0. Availabe online: http://leitlinienprogramm-onkologie.de/Lungenkarzinom.98.0.html (accessed on 23 February 2022).

34. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, D.K., AWMF). S3-Leitliniezur Diagnostik,Therapie und Nachsorge des Melanoms, Version 3.3. Availabe online: http://www.leitlinienprogramm-onkologie.de/leitlinien/melanom/ (accessed on 23 February 2022).

35. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, D.K., AWMF). S3-Leitlinie Diagnostik, Therapie und Nachsorge des Nierenzellkarzinoms, Langversion 3.0. Availabe online: https://www.leitlinienprogramm-onkologie.de/leitlinien/nierenzellkarzinom/ (accessed on 23 February 2022).

36. Disis, M.L.; Patel, M.R.; Pant, S.; Hamilton, E.P.; Lockhart, A.C.; Kelly, K.; Beck, J.T.; Gordon, M.S.; Weiss, G.J.; Taylor, M.H.; Chaves, J.; Mita, A.C.; Chin, K.M.; Heydebreck, A.v.; Cuillerot, J.-M.; Gulley, J.L. Avelumab (MSB0010718C; anti-PD-L1) in patients with recurrent/refractory ovarian cancer from the JAVELIN Solid Tumor phase Ib trial: Safety and clinical activity. *J Clin Oncol* **2016**, *34*, 5533-5533, doi:10.1200/JCO.2016.34.15_suppl.5533.

37. Hamanishi, J.; Mandai, M.; Ikeda, T.; Minami, M.; Kawaguchi, A.; Murayama, T.; Kanai, M.; Mori, Y.; Matsumoto, S.; Chikuma, S.; Matsumura, N.; Abiko, K.; Baba, T.; Yamaguchi, K.; Ueda, A.; Hosoe, Y.; Morita, S.; Yokode, M.; Shimizu, A.; Honjo, T.; Konishi, I. Safety and

Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. *J Clin Oncol* **2015**, *33*, 4015-4022, doi:10.1200/jco.2015.62.3397.

38. Varga, A.; Piha-Paul, S.A.; Ott, P.A.; Mehnert, J.M.; Berton-Rigaud, D.; Johnson, E.A.; Cheng, J.D.; Yuan, S.; Rubin, E.H.; Matei, D.E. Antitumor activity and safety of pembrolizumab in patients (pts) with PD-L1 positive advanced ovarian cancer: Interim results from a phase Ib study. *J Clin Oncol* **2015**, *33*, 5510-5510, doi:10.1200/jco.2015.33.15_suppl.5510.

39. Matulonis, U.A.; Shapira-Frommer, R.; Santin, A.D.; Lisyanskaya, A.S.; Pignata, S.; Vergote, I.; Raspagliesi, F.; Sonke, G.S.; Birrer, M.; Provencher, D.M.; Sehouli, J.; Colombo, N.; González-Martín, A.; Oaknin, A.; Ottevanger, P.B.; Rudaitis, V.; Katchar, K.; Wu, H.; Keefe, S.; Ruman, J.; Ledermann, J.A. Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study. *Ann Oncol* **2019**, *30*, 1080-1087, doi:10.1093/annonc/mdz135.

40. Drew, Y.; Kaufman, B.; Banerjee, S.; Lortholary, A.; Hong, S.; Park, Y.; Zimmermann, S.; Roxburgh, P.; Ferguson, M.; Alvarez, R. Phase II study of olaparib+ durvalumab (MEDIOLA): Updated results in germline BRCA-mutated platinum-sensitive relapsed (PSR) ovarian cancer (OC). *Annals of Oncology* **2019**, *30*, v485-v486.

41. Konstantinopoulos, P.A.; Waggoner, S.; Vidal, G.A.; Mita, M.; Moroney, J.W.; Holloway, R.; Van Le, L.; Sachdev, J.C.; Chapman-Davis, E.; Colon-Otero, G.; Penson, R.T.; Matulonis, U.A.; Kim, Y.B.; Moore, K.N.; Swisher, E.M.; Färkkilä, A.; D'Andrea, A.; Stringer-Reasor, E.; Wang, J.; Buerstatte, N.; Arora, S.; Graham, J.R.; Bobilev, D.; Dezube, B.J.; Munster, P. Single-Arm Phases 1 and 2 Trial of Niraparib in Combination With Pembrolizumab in Patients With Recurrent Platinum-Resistant Ovarian Carcinoma. *JAMA Oncol* **2019**, *5*, 1141-1149, doi:10.1001/jamaoncol.2019.1048.

42. Kossaï, M.; Leary, A.; Scoazec, J.Y.; Genestie, C. Ovarian Cancer: A Heterogeneous Disease. *Pathobiology* **2018**, *85*, 41-49, doi:10.1159/000479006.

43. Mota, A.; S, S.O.; Moreno-Bueno, G. Insight updating of the molecular hallmarks in ovarian carcinoma. *EJC Suppl* **2020**, *15*, 16-26, doi:10.1016/j.ejcsup.2019.11.001.

44. Meza-Perez, S.; Randall, T.D. Immunological Functions of the Omentum. *Trends Immunol* **2017**, *38*, 526-536, doi:10.1016/j.it.2017.03.002.

45. Heindl, A.; Lan, C.; Rodrigues, D.N.; Koelble, K.; Yuan, Y. Similarity and diversity of the tumor microenvironment in multiple metastases: critical implications for overall and progression-free survival of high-grade serous ovarian cancer. *Oncotarget* **2016**, *7*, 71123-71135, doi:10.18632/oncotarget.12106.

46. Jimenez-Sanchez, A.; Memon, D.; Pourpe, S.; Veeraraghavan, H.; Li, Y.; Vargas, H.A.; Gill, M.B.; Park, K.J.; Zivanovic, O.; Konner, J.; Ricca, J.; Zamarin, D.; Walther, T.; Aghajanian, C.; Wolchok, J.D.; Sala, E.; Merghoub, T.; Snyder, A.; Miller, M.L. Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient. *Cell* **2017**, *170*, 927-938, doi:10.1016/j.cell.2017.07.025.

47. FDA-NIH Biomarker Working Group. *BEST (Biomarkers, EndpointS, and other Tools) Resource*; Silver Spring (MD): Food and Drug Administration (US); Co-published by National Institutes of Health (US), Bethesda (MD): 2016.

48. Davis, K.D.; Aghaeepour, N.; Ahn, A.H.; Angst, M.S.; Borsook, D.; Brenton, A.; Burczynski, M.E.; Crean, C.; Edwards, R.; Gaudilliere, B.; Hergenroeder, G.W.; Iadarola, M.J.; Iyengar, S.; Jiang, Y.; Kong, J.T.; Mackey, S.; Saab, C.Y.; Sang, C.N.; Scholz, J.; Segerdahl, M.; Tracey, I.; Veasley, C.; Wang, J.; Wager, T.D.; Wasan, A.D.; Pelleymounter, M.A. Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. *Nat Rev Neurol* **2020**, *16*, 381-400, doi:10.1038/s41582-020-0362-2.

49. Plotti, F.; Terranova, C.; Guzzo, F.; De Cicco Nardone, C.; Luvero, D.; Bartolone, M.; Dionisi, C.; Benvenuto, D.; Fabris, S.; Ciccozzi, M.; Di Donato, V.; Panici, P.B.; Angioli, R. Role of BRCA Mutation and HE4 in Predicting Chemotherapy Response in Ovarian Cancer: A Retrospective Pilot Study. *Biomedicines* **2021**, *9*, doi:10.3390/biomedicines9010055.

50. van Zyl, B.; Tang, D.; Bowden, N.A. Biomarkers of platinum resistance in ovarian cancer: what can we use to improve treatment. *Endocr Relat Cancer* **2018**, *25*, R303-R318, doi:10.1530/ERC-17-0336.

51. Hynes, R.O. Integrins: bidirectional, allosteric signaling machines. *Cell* **2002**, *110*, 673-687, doi:10.1016/s0092-8674(02)00971-6.

52. Arnaout, M.A.; Mahalingam, B.; Xiong, J.P. Integrin structure, allostery, and bidirectional signaling. *Annu Rev Cell Dev Biol* **2005**, *21*, 381-410, doi:10.1146/annurev.cellbio.21.090704.151217.

53. Bianconi, D.; Unseld, M.; Prager, G.W. Integrins in the Spotlight of Cancer. *Int J Mol Sci* **2016**, *17*, 2037, doi:10.3390/ijms17122037.

54. Ganguly, K.K.; Pal, S.; Moulik, S.; Chatterjee, A. Integrins and metastasis. *Cell Adh Migr* **2013**, *7*, 251-261, doi:10.4161/cam.23840.

55. Aziz, M.; Agarwal, K.; Dasari, S.; Mitra, A.A.K. Productive Cross-Talk with the Microenvironment: A Critical Step in Ovarian Cancer Metastasis. *Cancers (Basel)* **2019**, *11*, 1608, doi:10.3390/cancers11101608.

56. Kobayashi, M.; Sawada, K.; Kimura, T. Potential of Integrin Inhibitors for Treating Ovarian Cancer: A Literature Review. *Cancers (Basel)* **2017**, *9*, 83, doi:10.3390/cancers9070083.

57. Scalici, J.M.; Harrer, C.; Allen, A.; Jazaeri, A.; Atkins, K.A.; McLachlan, K.R.; Slack-Davis, J.K. Inhibition of alpha4beta1 integrin increases ovarian cancer response to carboplatin. *Gynecol Oncol* **2014**, *132*, 455-461, doi:10.1016/j.ygyno.2013.12.031.

58. Wantoch von Rekowski, K.; König, P.; Henze, S.; Schlesinger, M.; Zawierucha, P.; Januchowski, R.; Bendas, G. The Impact of Integrin-Mediated Matrix Adhesion on Cisplatin Resistance of W1 Ovarian Cancer Cells. *Biomolecules* **2019**, *9*, doi:10.3390/biom9120788.

59. Casey, R.C.; Burleson, K.M.; Skubitz, K.M.; Pambuccian, S.E.; Oegema, T.R., Jr.; Ruff, L.E.; Skubitz, A.P. Beta 1-integrins regulate the formation and adhesion of ovarian carcinoma multicellular spheroids. *Am J Pathol* **2001**, *159*, 2071-2080, doi:10.1016/s0002-9440(10)63058-1.

60. Naci, D.; Vuori, K.; Aoudjit, F. Alpha2beta1 integrin in cancer development and chemoresistance. *Semin Cancer Biol* **2015**, *35*, 145-153, doi:10.1016/j.semcancer.2015.08.004.

61. Zheng, W.; Ge, D.; Meng, G. Reversing microtubule-directed chemotherapeutic drug resistance by co-delivering alpha2beta1 inhibitor and paclitaxel with nanoparticles in ovarian cancer. *Cell Biol Int* **2020**, *44*, 610-620, doi:10.1002/cbin.11261.

62. Slack, R.J.; Macdonald, S.J.F.; Roper, J.A.; Jenkins, R.G.; Hatley, R.J.D. Emerging therapeutic opportunities for integrin inhibitors. *Nat Rev Drug Discov* **2021**, 10.1038/s41573-021-00284-4, 1-19, doi:10.1038/s41573-021-00284-4.

63. Raab-Westphal, S.; Marshall, J.F.; Goodman, S.L. Integrins as Therapeutic Targets: Successes and Cancers. *Cancers (Basel)* **2017**, *9*, doi:10.3390/cancers9090110.

64. Stupp, R.; Hegi, M.E.; Gorlia, T.; Erridge, S.C.; Perry, J.; Hong, Y.K.; Aldape, K.D.; Lhermitte, B.; Pietsch, T.; Grujicic, D.; Steinbach, J.P.; Wick, W.; Tarnawski, R.; Nam, D.H.; Hau, P.; Weyerbrock, A.; Taphoorn, M.J.; Shen, C.C.; Rao, N.; Thurzo, L.; Herrlinger, U.; Gupta, T.; Kortmann, R.D.; Adamska, K.; McBain, C.; Brandes, A.A.; Tonn, J.C.; Schnell, O.; Wiegel, T.;

Kim, C.Y.; Nabors, L.B.; Reardon, D.A.; van den Bent, M.J.; Hicking, C.; Markivskyy, A.; Picard, M.; Weller, M. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* **2014**, *15*, 1100-1108, doi:10.1016/s1470-2045(14)70379-1.

65. Valdembri, D.; Serini, G. The roles of integrins in cancer. *Fac Rev* **2021**, *10*, 45, doi:10.12703/r/10-45.

66. Miksch, R.C.; Hao, J.; Schoenberg, M.B.; Dotzer, K.; Schluter, F.; Weniger, M.; Yin, S.; Ormanns, S.; D'Haese, J.G.; Guba, M.O.; Werner, J.; Mayer, B.; Bazhin, A.V. Development of a reliable and accurate algorithm to quantify the tumor immune stroma (QTiS) across tumor types. *Oncotarget* **2017**, *8*, 114935-114944, doi:10.18632/oncotarget.22932.

5. Acknowledgement

First, I want to thank my doctoral advisor PD Dr. Barbara Mayer for her guidance, support and encouragement. She provided the idea and the equipment for the experiments, the research data of the sphero-ID-Study and her experience. She taught me patience, tenacity, ambition and how to publish scientific data.

I would like to thank Prof. Alexandr Bazin for the opportunity to conduct the experimental research at his department.

I was very fortunate to have had Friedi as a 'lab partner' all along. Neither of us could have finished without the other. Our skills and characteristics are complementary, together every problem can be solved.

I want to thank Maja Schorkowitz, Michael Pohr, Anton Stolp und Frank Arnold for the help and the data they provided for my research. Also, I was very lucky to have met a lot of supportive lab colleagues: Sascha, Sebastian, Niklas, Eileen, Micha, Nici, Sev.

And of course, I'm very thankful to have a great family, great friends (especially the ones called Muskeltiere) and the greatest future husband, who supported me during exciting years, listened to my moaning and always believed in me.