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REVIEW

Aberrant MAPK Signaling Offers Therapeutic Potential for Treatment of Ovarian Carcinoma

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Abstract: Ovarian cancer remains the most lethal gynecological malignancy worldwide due to lack of effective screening, vague early symptoms, poor description of biomarkers, and absence of effective treatment regimes. Epithelial ovarian carcinoma (EOC) is categorized into five distinct disease subtypes which collectively account for ~90% of ovarian carcinomas. Most women present at advanced stages contributing to a poor overall 5-year survival rate. Standard treatment for EOC is cytoreductive surgery and platinum-based chemotherapy; however, most patients suffer from recurrence and platinum-resistant disease, which highlights an urgent need for targeted therapy. The high frequency of molecular alterations affecting gain-of-function signaling through the RAS mitogen-activated protein kinase (MAPK) pathway in EOC has prompted pre-clinical and clinical efforts toward research into the effectiveness of MAPK pathway inhibition as a second-line treatment. The RAS/MAPK pathway is a highly conserved signal transduction cascade, often disrupted in cancer, that regulates tumorigenic phenotypes including cellular proliferation, survival, migration, apoptosis, and differentiation. Herein, the role of the MAPK pathway in EOC with emphasis on targetability of the pathway is described. Pre-clinical and clinical efforts to target MAPK signaling in EOC have identified several MAPK pathway inhibitors that offer efficacious potential for monotherapy and in combination with other compounds. Thus, inhibition of the RAS/MAPK pathway is emerging as a tractable strategy for treatment of ovarian cancer that may permit development of personalized therapy and improved prognosis for women challenged by this disease.

Keywords: RAS/MAPK, ovarian, serous, clear-cell, mucinous, endometrioid

Introduction

Ovarian cancer is the most lethal gynecological malignancy worldwide. Approximately 90% of malignant ovarian cancers are epithelial ovarian carcinoma (EOC) derived from the epithelial cells lining the ovarian surface, peritoneum, or fallopian tube.[1] Due to lack of effective screening methods, poor description of early biomarkers, and vague distinctive symptoms, most women present at advanced stages contributing to a poor overall 5-year survival rate around 30–40%.[1–3] Despite an initial response to standard therapy of cytoreductive surgery and platinum-based chemotherapy, most patients suffer disease recurrence following treatment.[4,5] Therefore, a personalized regimen or targeted therapy for treating ovarian carcinoma remains an urgent unmet need.

The RAS/MAPK pathway is an essential signaling module that regulates cellular proliferation, cell survival, and apoptosis, all of which are perturbed in cancer.[6,7] Somatic mutations leading to constitutive RAS activity are drivers of many lethal cancers including pancreatic, lung, colorectal, melanoma, and certain hematological cancers.[6–8] Present, but less commonly described are the pathogenesis and molecular genetic features of a subset of primary EOC with somatic mutations in RAS pathway components.[3]

The potential for therapeutic targetability of the RAS/MAPK pathway in ovarian cancer has been previously described.[9,10] Histological evidence demonstrated punctual mutations are prevalent in EOCs, establishing RAS pathway involvement in

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some types of ovarian cancers.[10,11] Herein, we describe the role of the RAS/MAPK pathway in EOC with emphasis on targetability of the pathway directing novel treatment regimes.

The RAS-MAPK Pathway Signal Transduction Cascade

The RAS mitogen-activated protein kinase (MAPK) pathway is a highly conserved signal transduction cascade that regulates cell growth, survival, migration, apoptosis, and differentiation.[6] Activation of the canonical MAPK cascade involves signaling through RAS, RAF, MEK, and extracellular signal-regulated kinase (ERK). Signals received at the cell surface activate receptor tyrosine kinases (RTKs), which are essential to promote activation of RAS and RAF followed by the sequential phosphorylation of MEK and ERK (Figure 1).[7] RAS/MAPK signaling is triggered by multiple growth factors, inflammatory cues, and cytokine stimulation, thus modulating diverse cellular and physiological processes.[12]

RAS is the prototypical member of the Ras family and is encoded by three proto-oncogenes, *HRAS, KRAS*, and *NRAS*, all of which are ubiquitously expressed in mammalian cells and interact with the same set of effector proteins.[6,8,13,14] RAS is the most frequently mutated gene in human cancer, highlighting an essential role in tumorigenesis.[8,14] Unfortunately, direct inhibitors of RAS have eluded researchers for more than three decades, earning RAS the title of "undruggable". Thus, finding therapeutically tractable targets in RAS effector pathways is critical for the effective treatment of RAS-driven cancers.

RAS proteins are small GTPases that operate as molecular switches to activate signal transduction pathways.[14] In the absence of oncogenic mutation, RAS cycles between inactive GDP-bound and active GTP-bound forms. Guanine nucleotide exchange factors (GEFs) promote exchange of GDP for GTP leading to RAS activation.[15] RTK activation stimulates GEF activity and promotes GDP exchange on RAS permitting RAS to interact directly with RAF. Intrinsic but weak GTPase activity of RAS is augmented by a class of tumor suppressors called RAS GTPase activating proteins (GAPs), that catalyze the hydrolysis of GTP and thus inactivate RAS.[6,15,16] RAS signaling is coupled to the activation of multiple effector pathways including RAF/MEK/ERK and the phosphoinositide 3-kinase (PI3K)/AKT that relay mitogenic signaling to regulate pro-tumorigenic phenotypes (Figure 1).

Commonly observed in cancer, gain-of-function mutations trap RAS in a constitutively activated, GTP-bound state potentiating MAPK signaling. Mutations in codons 12, 13, or 61 of RAS disrupt GAP-mediated GTP hydrolysis, allowing these mutants to accumulate in a persistently GTP-bound state.[8,14] For RAS-driven cancers, the lack of RAS

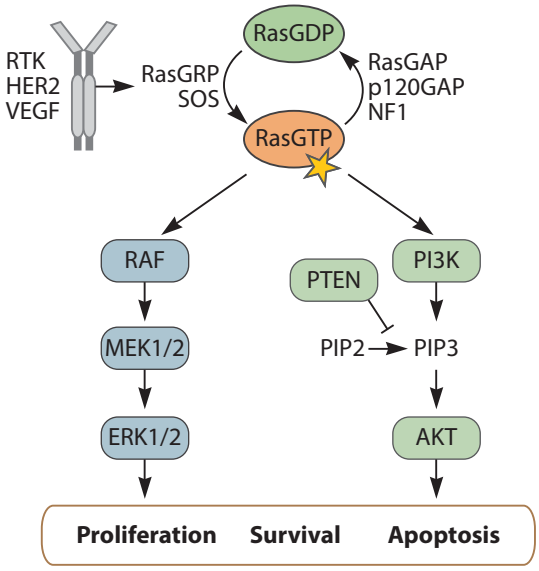

Figure 1 RAS signaling engages MAPK and PI3K pathways. Receptor tyrosine kinase (RTK, e.g. HER2, VEGF) activation by mitogenic signaling activates guanine exchange factors (e.g. RasGRP, SOS) that promote exchange of GDP for GTP on Ras. GTPase activating proteins (e.g. RasGAP, p120GAP, NF1) facilitate hydrolysis of GTP to inactivate Ras. Oncogenic mutation (yellow star) traps Ras in a constitutively active state. Activated Ras engages downstream effectors RAF and PI3K to regulate signaling through the MAPK (left) and PI3K (right) pathways affecting pro-tumorigenic phenotypes such as proliferation, survival, and apoptosis.

mutant specific therapeutics underscores an urgent need to identify therapeutic strategies targeting RAS effectors, which may demonstrate selectivity and improved outcomes over conventional chemotherapy.[10,17]

The Dichotomy of Ovarian Carcinoma: Type I and Type II Cancer

EOC are a heterogeneous group of cancers that account for ~90% of all ovarian cancers.[1,18,19] Although EOC share a common site of tumor growth in the ovary, evidence suggests EOC encompasses five distinct diseases, with differing clinical presentation, genetic background, response to chemotherapy, prognosis, and sites of origin.[19–21] The major histologically distinct subtypes include serous high- and low-grade, clear cell, endometrioid, and mucinous carcinomas, each characterized by specific genetic backgrounds (Figure 2A). Importantly, the molecular alterations that define subtypes of EOC offer the potential for targeted therapies including those subtypes that may respond to RAS/MAPK inhibition.[20]

Studies suggest a dualistic model of ovarian carcinogenesis that correlates with the clinical, histopathological, genetic, and molecular features of the disease. In this model, ovarian tumors are divided into two groups designated as type I and type II.[22] Based on molecular and cell of origin studies, type II tumors can be classed together; however, type I tumors are not homogeneous, even within distinct histological subtypes arguing for a refinement of the classification.[23,24] Type I cancers include low-grade serous ovarian carcinoma (LGSOC), clear cell, endometrioid, and mucinous carcinomas, whereas type II cancers contain high-grade serous ovarian carcinoma (HGSOC), undifferentiated carcinoma and

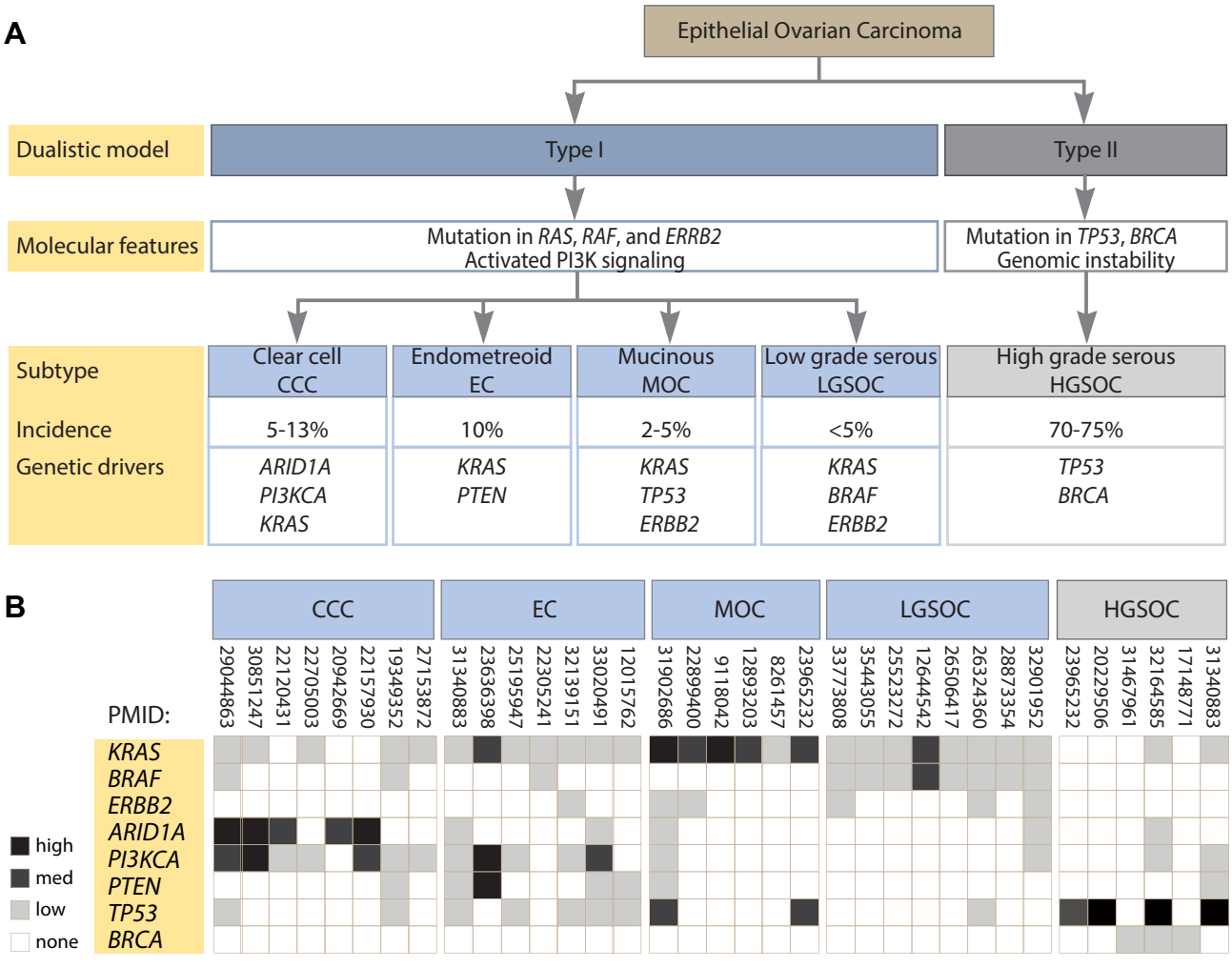

Figure 2 Analysis of genetic aberrations in epithelial ovarian carcinoma. (**A**) Classification of epithelial ovarian cancers using the type I and type II dualistic approach into five distinct subtypes based on genetic abnormalities and histopathology. (**B**) Heat map presentation of frequency of mutation for each of the indicated genes in the five subtypes of epithelial ovarian cancer. Percentage of mutation frequency reported in the indicated references was converted to high (>80%), medium (40–80%), and low (<40%) expression value. None may refer to zero or not reported. References indicated as PubMed reference number (PMID).

malignant mixed mesodermal carcinosarcoma.[1] Although HGSOC and LGSOC best fit the description of the dualistic model, endometrioid, mucinous, and clear cell type I tumors include an uncertain origin, heterogeneous mutational spectrum, and clinical behavior, thus complicating classification.[24]

Type I tumors are often diagnosed at an early stage, are slow growing, generally confined to the ovary at diagnosis, and develop from well-established precursor lesions termed "borderline" tumors.[25] Often resisting conventional chemotherapy, type I tumors are genetically stable with wild-type *TP53* and *BRCA1*/*BRCA2*. Type I tumors are frequently characterized by mutations affecting the RAS/MAPK pathway including *KRAS, BRAF*, and *ERBB2* (HER2/neu), as well as having aberrant activation of the PI3K/AKT pathway, likely associated with increased RAS activity and mutation or loss of *PTEN*.[9,25] In contrast, type II tumors are rapidly growing highly aggressive neoplasms for which well-defined morphological precursor lesions have not been described.[22] Type II tumors have a high level of genomic instability, are nearly ubiquitous for mutations in *TP53*, and nearly half harbor *BRCA1*/*BRCA2* mutations.[22,25,26] Although type II tumors typically present with wild-type RAS genes, type I tumors frequently harbor gain-of-function RAS mutations critical to delineate predictive therapeutic decisions.[22,25,27]

Pathogenic Features of EOC Subtypes: Targeting the RAS/MAPK Pathway

Evidence from mutational analysis of EOC tumors, cell lines, and micro-dissected tumors have established the mutational frequency of RAS/MAPK genes associated with the pathogenesis of EOC (Figure 2B). Analysis of the clinical features, frequency of mutations in genes affecting RAS pathway activity, and the potential for targeting the MAPK in studies supported by pre-clinical and clinical research are described below for each EOC subtype (Figure 3).

High-Grade Serous Carcinoma (HGSOC)

HGSOC is the most common histological subtype of EOC representing ~70% of cases, presenting at an advanced stage, and accounting for the most deaths from ovarian cancer.[9] HGSOC is typically driven by copy number abnormalities and genomic instability.[9] Evidence suggests that HGSOC tumors originate in fallopian tubal secretory epithelial cells (FTSEC) and serous tubal intraepithelial carcinoma (STIC) has been established as the precursor lesion for HGSOC in animal models, leading to progression of advanced stage disease.[26,28]

TP53 mutations occur in 96% of HGSOC presenting more frequently in advanced ovarian carcinomas and mutations in *BRCA1*/*BRCA2* are also frequently observed.[26] Early *TP53* mutation and BRCA loss cause deficiencies in DNA repair

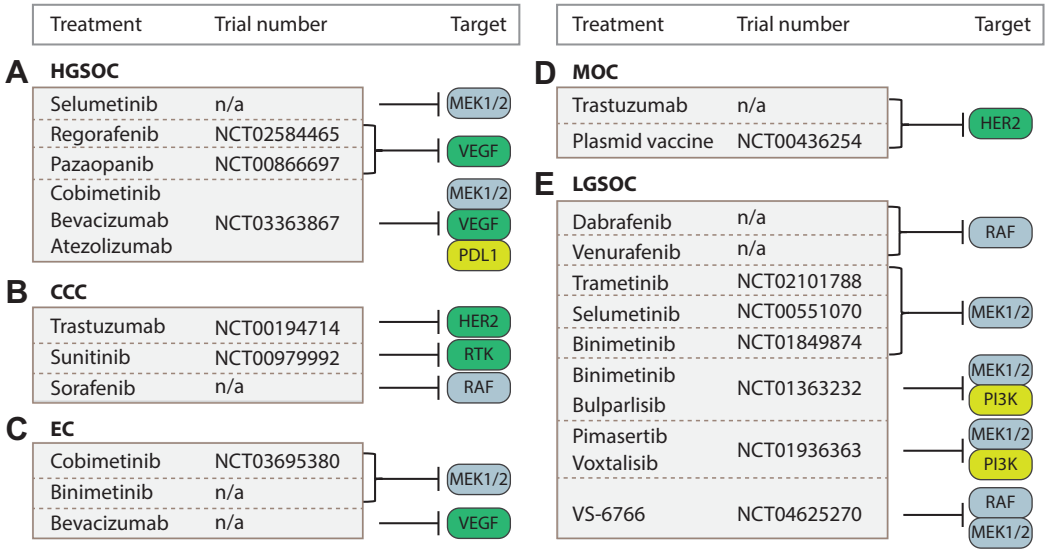

Figure 3 Treatment strategies for epithelial ovarian carcinoma. (**A**–**E**) Treatment regimes described in the text for subtypes of epithelial ovarian cancer in (**A**) high-grade serous HGSOC, (**B**) clear cell carcinoma CCC, (**C**) endometrioid EC, (**D**) mucinous MOC, and (**E**) low-grade serous LGSOC with the compounds listed. Compounds in clinical trials are shown with the associated clinical trial numbers from the ClinicalTrials.gov database if applicable. Not available (n/a) refers to compounds involved in studies without clinical support. Molecular targets of each compound affecting RAS/MAPK pathway (blue), receptors (green), and other targets (yellow) are shown.

pathways which trigger chromosomal instability and widespread somatic copy number changes.[21] Genetic mutations driving HGSOC oncogenesis are supported by a genetically engineered mouse model demonstrating *Pax8*, a marker of the fallopian secretory cells, which drove Cre-mediated inactivation of *Brca1, Brca2, Tp53*, and *Pten*, transformed fallopian tube epithelial cells which led to the development of STIC lesions.[28]

In some HGSOC tumors, oncogenesis appears to be driven by amplification of genes that activate the RAS/MAPK and PI3K/AKT pathways.[9] One study has extensively characterized *KRAS* and *BRAF* mutations in 264 ovarian neoplasms and identified *KRAS* mutations in up to 12% of HGSOC tumors.[29] Although RAS mutation rates are low in HGSOC, high transcript levels of *RAF1, ERK1*, and *ERK2* have been significantly correlated with poor platinum-free survival in patients with advanced stage disease.[30] Analysis of differential gene expression in 408 HGSOC tumors from the cancer genome atlas demonstrated a high intratumor MAPK-activated gene set was prognostic for poor outcome and decreased survival.[31] The study demonstrated that selumetinib (a MEK inhibitor) reversed expression of a subset of MAPK-activated genes in vitro and in xenografts, suggesting the gene signature was predictive of a MEK inhibitor response.[31]

For HGSOC, cytoreductive surgery and combination chemotherapy with platinum compounds and taxanes is the first-line treatment,[32] but due to high incidence of resistant disease, secondary treatments must be explored (Figure 3A). Although chemotherapy with carboplatin and paclitaxel achieves complete response, platinum resistance occurs at an alarming rate, with disease recurrence observed in 75% of HGSOC patients, thus contributing to an abysmal 5-year survival rate.[9] Molecular characterization of platinum-resistant HGSOC has identified activation of pro-proliferative signaling pathways, including the RAS/MAPK in disease resistance.[30] In resistant tumors, crosstalk drives numerous downstream targets of RAS/MAPK signaling including activation of ERK regulated transcription factors leading to upregulation of pro-tumorigenic gene signatures. In cisplatin-resistant HGSOC cell lines, resistance was attributed to activation of mutant p53 by MEK phosphorylation, arguing that MEK inhibition may be a therapeutic option for refractory HGSOC.[33]

Vascular endothelial growth factor (VEGF) is a membrane receptor that regulates a complex signaling cascade involving multiple kinases including the RAS/MAPK pathway to stimulate angiogenesis. Anti-angiogenesis agents including those that target VEGF are being investigated as second-line therapy for recurrent HGSOC, since high VEGF expression has been observed in serous carcinomas and most of these tumors are dependent on VEGF for progression.[34] Regorafenib, a multikinase inhibitor that blocks signaling through VEGF receptors and the MAPK pathway,[35] is currently in a phase II clinical trial for anti-tumor activity against persistent ovarian carcinoma in patients harboring tumors that are predominantly HGSOC. Unfortunately, no significant difference has been noted in progression-free survival (PFS), objective response rates (ORR), or overall survival (OS) between regorafenib or tamoxifen treatment groups (Table 1: NCT02584465). A study in phase III has examined pazopanib (a VEGF inhibitor) in patients with advanced ovarian cancer. Although pazopanib prolonged PFS to 17.9 months compared with 12.3 months in placebo, there was no associated improvement in median OS (Table 1: NCT00866697).

Additional evidence for VEGF inhibition as a treatment for HGSOC comes from a clinical trial that has tested cobimetinib (a MEK inhibitor) in combination with bevacizumab (a VEGF inhibitor) and PD-L1 inhibition. The PD-L1 pathway regulates adaptive immune resistance often exerted by tumor cells in response to anti-tumor activity.[36] MEK inhibition with cobimetinib in combination with a monoclonal antibody inhibiting PD-L1 (atezolizumab) has previously been used to treat patients with solid tumors and shown to have potential synergistic activity in metastatic colon cancer.[37] Referred to as the ABC study, the triple combination of atezolizumab, bevacizumab, and cobimetinib is being explored in a phase II clinical trial for treatment of recurrent platinum-resistant HGSOC (Table 1: NCT03363867). Cobimetinib is FDA-approved for use in combination with a BRAF inhibitor vemurafenib for the treatment of advanced melanomas harboring *BRAF* (*V600E/K*) mutation, suggesting additional combination therapies could be explored for ovarian cancer.

Clear Cell Carcinoma (CCC)

CCC is the second most common type of epithelial ovarian cancer and accounts for approximately 5–13% of EOC.[38–40] CCC has been reported to arise from endometriosis that has implanted on the ovaries or in the peritoneal cavity and undergone malignant transformation. Research suggests that endometriosis may be the precursor of CCC and is thought to develop from retrograde menstruation.[22] CCC frequently presents at an early stage with a high propensity for recurrence after primary chemotherapy.[40] Patients with early-stage disease who underwent platinum-based therapy

Table 1 Summary of Clinical Trials Targeting MAPK Signaling in Ovarian Carcinoma

Target	Therapeutic	Cancer Type	Title	Date	Trial Number
BRAF MEK	Niraparib Cobimetinib	Advanced solid tumors	A Phase Ib Study of Cobimetinib Administered in Combination With Niraparib, With or Without Atezolizumab, To Patients With Advanced Platinum-sensitive Ovarian Cancer	2018–2022	NCT03695380
RAF MEK FAK	VS-6766 Defactinib	LGSOC	A Phase II Study of VS-6766 (Dual RAF/MEK Inhibitor) Alone and In Combination With Defactinib (FAK Inhibitor) in Recurrent Low-Grade Serous Ovarian Cancer (LGSOC)	2020–2022	NCT04625270
MEK	Selumetinib	Ovarian, fallopian tube, or peritoneal carcinoma	Selumetinib Sulfate in Treating Woman With Recurrent Low-Grade Ovarian Cancer or Peritoneum Cancer	2007–2020	NCT00551070
MEK PI3K	Binimetinib Bupalisib	Advanced solid tumors	A Phase Ib, Open-label, Multi-center, Dose-escalation and Expansion Study of an Orally Administered Combination of BKM120 Plus MEK162 in Adult Patients With Selected Advanced Solid Tumors	2011–2020	NCT01363232
MEK MTOR/ PI3K	Pimasertib Voxtalisib	LGSOC, Peritoneal carcinoma, serous borderline ovarian	Trial of Pimasertib With SAR245409 or Placebo in Ovarian Cancer	2013–2018	NCT01936363
MEK	Cobimetinib	EOC	A Phase II, Open-Label, Multicenter, Platform Study Evaluating the Efficacy and Safety of Biomarker-Driven Therapies in Patients With Persistent or Recurrent Rare Epithelial Ovarian Tumors	2021–2024	NCT04931342
MEK	Binimetinib	LGSOC, peritoneal carcinoma, fallopian carcinoma	A Study of MEK162 vs Physician's Choice Chemotherapy in Patients With Low-grade Serous Ovarian, Fallopian Tube or Peritoneal Cancer	2013–2022	NCT01849874
MEK	Trametinib	LGSOC	Trametinib in Treating Patients With Recurrent or Progressive Low-Grade Ovarian Cancer or Peritoneal Cavity Cancer	2014–2022	NCT02101788
MEK VEGF PDL1	Cobimetinib Bevacizumab Atezolizumab	HGSOC	BEACON - A Phase II Study of Bevacizumab, Atezolizumab and Cobimetinib in Patients With Recurrent Platinum Resistant High Grade Serous Ovarian Cancer	2017–2019	NCT03363867
ERK AKT	ONC201 Paclitaxel	Ovarian, Fallopian tube, or peritoneal carcinoma	ONC201 Plus Weekly Paclitaxel in Patients With Platinum Refractory or Resistant Ovarian Cancer	2019–2022	NCT04055649
ERK	Ulixertinib	Advanced solid tumors	Phase I Dose-Escalation, Safety, Pharmacokinetic and Pharmacodynamic Study of BVD-523 in Patients With Advanced Malignancies	2013–2020	NCT01781429
ERK	Rineterkib	Advanced solid tumors	A Phase I Dose Finding Study of Oral LTT462 in Adult Patients With Advanced Solid Tumors Harboring MAPK Pathway Alterations.	2016–2019	NCT02711345
VEGF	Sunitinib	CCC	Sunitinib Malate in Treating Patients With Persistent or Recurrent Clear Cell Ovarian Cancer	2009–2020	NCT00979992

(*Continued*)

Table 1 (Continued).

Target	Therapeutic	Cancer Type	Title	Date	Trial Number
VEGF	Pazopanib	Ovarian, fallopian tube, or primary peritoneal cancer	A Phase III Study to Evaluate the Efficacy and Safety of Pazopanib Monotherapy Versus Placebo in Women Who Have Not Progressed After First Line Chemotherapy for Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer	2009–2021	NCT00866697
VEGF	Regorafenib	Ovarian carcinoma, all subtypes	A Randomized, Open-label, Comparative, Multicenter, Phase II Study of the Efficacy and Safety of Regorafenib Versus Tamoxifen in Patients with Platinum-sensitive Ovarian Carcinoma and Isolated Biological Progression	2015–2021	NCT02584465
HER2	Cytotoxic T Cells	HER2-positive breast or ovarian cancer	A Phase I Study of a DNA Plasmid Based Vaccine Encoding the HER-2/Neu Intracellular Domain in Subjects With HER-2/Neu (HER2) Overexpressing Tumors	2005–2021	NCT00194714
HER2	HER2/Neu vaccine	HER2-positive breast or ovarian cancer	A Phase I Study of a DNA Plasmid Based Vaccine Encoding the HER-2/Neu Intracellular Domain in Subjects With HER-2/Neu (HER2) Overexpressing Tumors	2007–2022	NCT00436254
HER2	Trastuzumab	HER-positive solid tumors	ISPY-P1.01:Evaluating the Safety of Weekly Paclitaxel With Trastuzumab Duocarmazine (SYD985) in Patients With Metastatic Cancer	2020–2021	NCT04602117
HER2	DP303c	Advanced ovarian cancer	An Open-label, multicentre, Phase II Study of DP303c Injection in Patients With HER2-expressing Advanced Ovarian Cancer	2021–2021	NCT04828616
KRAS	siRNA	PDAC	Exosomes in Treating Participants With Metastatic Pancreas Cancer With KrasG12D Mutation	2018–2022	NCT03608631

corroborate a high recurrence rate and decreased survival.[41] Since CCC display aggressive clinical phenotypes which rarely respond to platinum-based chemotherapy, prognosis is reported as worse than in HGSOC.[41,42]

The majority of CCC express wild type *TP53* and rarely harbor germline mutations in *BRCA1*/*BRCA2*. Consequently, CCC show low levels of chromosomal instability with fewer copy number variants.[43,44] Collectively, the most common molecular aberrations involve *ARID1A*, the PI3K/AKT pathway, and RTK signaling through the RAS/MAPK pathway.[44] Somatic inactivating mutation of *ARID1A* is reported to occur in up to 50% of CCC cases.[21,22] The *ARID1A* gene encodes a protein involved in chromatin remodeling which functions as a tumor suppressor in ovarian clear cell carcinogenesis.[45] In addition, approximately 50% of CCC harbor inactivating *PTEN* mutations and an activating mutation of *PIK3CA*, the catalytic subunit of PI3K, in up to 40% of cases.[21,22] *KRAS* mutations have been identified in 15–30% of CCC with mutations specifically located in codon 12 but not in codon 13.[46,47]

Currently, the preferred treatment for CCC is a complete resection of the tumor which can be complicated in advanced stage disease.[9] Further, drug resistance contributes to the poor prognosis of patients with CCC. An in vitro study revealed that low clear cell proliferation may account for chemoresistance to cisplatin via mechanisms that include decreased drug accumulation, increased drug detoxification, and increased DNA repair activity.[42] Additional treatment options are thus being investigated (Figure 3B).

CCC has been reported to express actionable levels of *ERBB2* pointing to a potential role for HER2 inhibitors in treatment.[3] In ongoing clinical trials, *HER2* positive ovarian cancer patients are being treated with trastuzumab, a monoclonal antibody against HER2 (Table 1: NCT00194714) and in other ongoing studies, antibody-drug conjugates targeting HER2 are being investigated in advanced solid tumors (Table 1: NCT04602117 and NCT04828616).

A case study reported advanced chemo-resistant CCC that was also resistant to PI3K/AKT pathway inhibitors despite harboring a *PIK3CA* mutation.[48] The tumor responded to treatment with combination therapy of sunitinib, a small-molecule with activity against multiple RTKs and sorafenib (a RAF-kinase inhibitor) which suggested that blocking MAPK signaling in these tumors could be a targetable option.[48] A phase II clinical study in patients with recurrent CCC demonstrated sunitinib monotherapy had minimal activity in persistent or recurrent CCC with 2 of 35 (6.7%) patients showing a complete or partial response (Table 1: NCT00979992). Combination treatments should be explored in this subtype.

Endometroid Carcinoma (EC)

EC is tied with clear cell as the second-most common EOC subtype, accounting for approximately 10% of cases.[39] EC typically presents as a unilateral mass confined to the ovary, which is difficult to distinguish from HGSOC, especially in patients with solid tumors.[38] Overall survival is a promising 10 years when tumors primarily display endometrioid histology.[49]

EC is frequently associated with endometrioid borderline tumors and like CCC, has been suggested to arise from endometriosis that has implanted on the ovaries or in the peritoneal cavity and undergone malignant transformation.[49,50] Endometriosis is a benign disease that is considered a precancerous lesion with potential for metastasis to the colon or lung.[49,51] Endometriosis can affect up to 15% of women and those with a history of endometriosis are at increased risk of ovarian cancer.[51]

KRAS is mutated in 46% of ovarian endometrioid cases, with *KRAS* (*G12V*) mutant allele most frequently detected.[52] The PI3K/AKT pathway has been implicated in development of chemotherapy resistance in EC.[53] EC typically presents with somatic mutations in *ARID1A, CTNNB1, PI3KCA*, and *PTEN*.[1,21,38] Mutation of *ARID1A* and *CTNNB1* occurs in 30% of cases.[21] The *CTNNB1* gene encodes for beta-catenin, an essential component of the Wnt signaling pathway which regulates cell growth and proliferation. Mutations in *CTNNB1* are also linked to colorectal cancer, medulloblastoma, and ovarian cancer.

Analysis of underlying genetic changes demonstrated mutations in *PTEN* (32%), *TP53* (17%), *CTNNB1* (13%), and *KRAS* (13%) in 53 sporadic EC tumors.[54] Molecular stratification of 112 EC cases using whole exome sequencing provided robust assessment of the mutational landscape of EC detecting mutation in *CTNNB1* (43%), *PIK3CA* (43%), *ARID1A* (36%), *PTEN* (29%), *KRAS* (26%), *TP53* (26%), and *SOX8* (19%) correlating with previous results.[55] Upon examination of the frequency of these mutations in clinically distinct EC subtypes, cases with *TP53* mutation demonstrated greater genomic complexity concurrent with decreased survival, and cases with *CTNNB1* mutation, which was mutually exclusive with *TP53* mutation, demonstrated low genomic complexity and better clinical outcome. Moreover, high frequency of mutations in the RAS/MAPK and PI3K/AKT pathways emerged suggesting therapeutic potential.

PTEN mutations are common in EC but less common in other subtypes of ovarian cancer. PTEN is a tumor suppressor frequently found to be mutated or deleted in a wide range of human cancers which drives dysregulated signaling through the PI3K/AKT pathway.[56] Conditional inactivation of *Pten* in mice resulted in formation of adenocarcinomas morphologically like human EC with 100% penetrance, short latency, and rapid progression toward metastatic disease.[57] The murine model supports the idea that inactivation of *PTEN* is an early event in EC tumorigenesis. Genetic mouse models of endometriosis and EC have been described based on the expression of oncogenic *Kras* or conditional *Pten* deletion within the ovarian surface epithelium. Both models gave rise to preneoplastic ovarian lesions suggesting endometriosis may be the cause of malignant transformation leading to ovarian cancer.[58] The combination of *Kras* mutation and conditional *Pten* deletion led to the induction of invasive metastatic endometrioid ovarian adenocarcinomas with complete penetrance.[58]

Treatment for EC is typically cytoreduction therapy followed by chemotherapy which may improve survival and decrease recurrence; however, following first-line platinum therapy many patients relapse with resistant disease.[59,60] Although genetic models have clearly demonstrated oncogenic *KRAS* induces endometriosis and may lead to the development of invasive EC, treatment options for EC targeting RAS effectors such as RAF and MEK are lagging (Figure 3C). Currently, cobimetinib, a MEK inhibitor described above for use in HGSOC, is in an ongoing phase I clinical trial evaluating combination with a poly ADP-ribose polymerase (PARP) inhibitor in patients with high grade serous or EC (Table 1: NCT03695380).

Targeting the MAPK pathway has been examined in patient-derived xenograft (PDX) models from platinum-relapsing endometrioid tumors, chosen based on the presence of activated RAS, mutant *TP53*, lack of *PTEN*, and activation of the PI3K pathway.[60] The study employed a combination regimen containing binimetinib (a MEK inhibitor), bevacizumab (a VEGF inhibitor), and paclitaxel as second-line therapy. The triple combination induced long-lasting response without increased toxicity better than dual combinations, demonstrating MEK inhibition can augment anti-tumor activity of the other compounds.[60]

Mucinous Ovarian Cancer (MOC)

MOC is a rare histological subtype representing less than 5% of all EOC.[22] Mucinous tumors have a multicystic structure with more than 90% of cells filled with conspicuous amounts of mucin. Mucinous ovarian tumors are postulated to arise from benign lesions, which transform into mucinous borderline tumors, and then into invasive cancer. MOCs morphologically resemble adenocarcinomas of the pancreas and gastrointestinal tract, complicating the differentiation of primary ovarian tumors from metastatic disease.[61] Tumor progression of mucinous carcinoma shows benign-appearing, borderline, non-invasive, and invasive components.[21] Patient outcomes and response to conventional chemotherapy are often poor.[38]

The most common genetic defects in mucinous tumors affect the RAS/MAPK pathway. More than 75% of mucinous ovarian tumors harbor activating *KRAS* mutations occurring predominantly at codon 12.[27,29,38,62] Other frequently mutated genes include *BRAF, TP53, PTEN, PI3KCA*, and *CDKN2A*.[38] *ERBB2* amplification is relatively common in MOC and is observed in 20–40% of cases.[63,64] *ERBB2* has been linked to enhanced survival of MOC patients and may be a potential biomarker.[65] In some tumors, mutation of *KRAS* and amplification of *ERBB2* have been observed in the same cell populations, with consistent *KRAS* allelic frequency in both *ERBB2* amplified and non-amplified regions, suggesting *KRAS* mutation occurred in advance of the amplification event.[21,63,66]

TP53 mutations are common and occur concomitantly with *KRAS* mutations in MOC in 36% of cases.[67] Ovarian cells sourced from *Pten/Kras (G12D)* mice develop serous EOC with 100% penetrance.[68] When the *Pten/Kras (G12D)* mutant mouse strain was crossed with mutant *Tp53(R172H)* heterozygous mice, resulting *WT/Tp53(R172H)* mice presented with mucinous cystadenocarcinomas at 12 weeks of age, recapitulating human mucinous ovarian tumors.[68] Although tumors were derived from *Pten/Kras* (*G12D*) and promoted EOC, differential effects on disease features and progression depended on the presence or absence of the wild-type *TP53* allele. This model provides genetic evidence that mutant *TP53* promotes EOC differentiation and metastasis.

MOC is frequently resistant to conventional platinum-based chemotherapy with low response rates and is unlikely to respond to PARP inhibition commonly used to treat other EOC cases.[9,64] Due to the high frequency of activating *KRAS* mutations, the RAS/MAPK pathway has been suggested as a therapeutic target.[2] However, perhaps due to the rarity of MOC, targeted therapies against downstream RAS pathway components have not been explored clinically (Figure 3D). However, some research suggests targeting HER2 is a viable option for *ERBB2* amplified advanced or recurrent MOC.[63,66] In one study, three patients with MOC displaying *ERRB2* amplification received trastuzumab in combination with conventional chemotherapy and one patient showed a positive response.[69] In addition, a current phase I trial is addressing the safety of a plasmid-based vaccine directed against the ICD of HER2 in combination with an immunostimulatory agent, in patients with *HER2* amplified advanced breast or ovarian cancers to stimulate HER2-specific immune responses (Table 1: NCT00436254).

Low-Grade Serous Carcinoma (LGSOC)

LGSOC is the rarest subtype of EOC accounting for less than 5% of all EOC cases.[39,70] LGSOC is typically diagnosed in younger women and presents as a distinct pathologic and clinical entity characterized by less aggressive behavior compared with HGSOC, a more indolent growth pattern, and poor response to systemic therapy compared with HGSOC.[2,9,26] LGSOC may arise from serous tumors with low malignant potential called borderline serous carcinomas or *de novo* from the ovary or peritoneum.[9,71] Advanced LGSOC is associated with poor long-term prognosis.[72]

Although LGSOC tumors evolve slowly, evidence suggests limited benefit of cytotoxic therapy due to high resistance of LGSOC to platinum-based neoadjuvant chemotherapy.[71] Surgical cytoreduction is associated with improved PFS and

OS in women with advanced-stage disease.[73] However, high risk of recurrence and cancer-related death is a major concern. Because LGSOC is relatively chemo-resistant, the need for targeted therapies is an urgent demand.

LGSOC appears to be driven by RAS/MAPK pathway activation with wild type *TP53* on a background of a relatively normal karyotype.[9] Most low-grade tumors typically harbor *KRAS* mutation in 30–50% of cases or *BRAF* mutation in 15–40% of cases.[21,27,29,74–76] Somatic mutation of *KRAS* in codons 12 and 13 and *BRAF* mutation in codon 599 are most common.[77] Activating *NRAS* (*Q61R*) mutation has been detected in 4% of LGSOC tumors, indicating that *NRAS* plays a limited role in this subtype.[78] Copy number aberrations have been observed with loss of 9p and homozygous deletions of the *CDKN2A/2B* locus.[79]

Somatic ovarian cells derived from *Tp53* deficient mice can be transformed by oncogenic *Kras* demonstrating the ovarian surface epithelium (OSE) is the precursor tissue for ovarian carcinomas.[80] In a mouse model of LGSOC, expression of *Kras* (*G12D*) in OSE cells with loss of *Pten* induced low-grade ovarian serous papillary adenocarcinomas at an early age with 100% penetrance.[81] Transformation of immortalized human ovarian serous cystadenoma cells by oncogenic *KRAS* (*G12V*) and *PIK3CA* have been shown to drive development of tumors that histologically resemble LGSOC.[82] Analysis of oncogenic *KRAS* gene signature datasets from human ovarian tumors and the OSE ovarian mouse model documented significant overlap, consistent with an essential role of RAS in transformation.

In contrast to HGSOC, LGSOC shows a low response rate to cytotoxic chemotherapy.[83] Like other type I cancers, LGSOC responds poorly to platinum-based chemotherapy due to the high frequency of RAS/RAF mutations suggesting patients with LGSOC may derive clinical benefit from MAPK-targeted therapies.[9,26] Many studies confirm that LGSOC can be defined by somatic mutations in RAS which points to novel treatment paradigms.[84] Recurrent or persistent LGSOC following prior platinum-based chemotherapy is the subject of ongoing evaluation with BRAF and MEK inhibitors in clinical trials (Figure 3E).

Response to BRAF inhibitors has been reported in case studies of LGSOC. Partial and durable responses following BRAF inhibition with dabrafenib or vemurafenib have been observed in patients with *BRAF* (*V600E*) mutant LGSOC.[74,85,86] Additional studies have demonstrated partial responses to BRAF inhibitors when combined with MEK inhibitors. Patients with LGSOC harbouring a *BRAF* (*V600E*) mutation developed sustained clinical response with combination treatment of dabrafenib and trametinib, an allosteric MEK1/2 inhibitor.[87] The combination of both drugs mitigated tumor-acquired resistance and decreased the incidence of secondary malignancies. However, research has not established if combination therapy with BRAF and MEK inhibitors is superior to monotherapy in *BRAF* mutated LGSOC.[87] Trametinib monotherapy is emerging as a promising treatment option for LGSOC with efficacy over chemotherapy.[84] PDX models of LGSOC that harbored *KRAS* (*G12V*) mutation showed effective sensitivity to trametinib.[88]

A phase II/III clinical trial on patients with LGSOC harboring activating *KRAS, NRAS*, or *BRAF* mutations demonstrated trametinib significantly improved PFS and ORR compared with standard chemotherapy in those with recurrent or persistent disease (Table 1: NCT02101788). The promising results from this trial have led to the inclusion of trametinib as a therapeutic option for LGSOC and provide the first strong evidence that LGSOC should be treated differently than HGSOC based on aberrant MAPK pathway activation.

Several other studies evaluating different MEK inhibitors in clinical trials for LGSOC have been conducted with varying degrees of success. In a phase II clinical trial, 15% of patients with recurrent LGSOC responded to selumetinib monotherapy which was four times that of cytotoxic chemotherapy.[89] Similarly, a single-arm phase II study in 52 patients with recurrent LGSOC demonstrated selumetinib was well-tolerated and showed a modest ORR with one complete response and seven partial responses. Notably, 35 patients (65%) achieved stable disease (Table 1: NCT00551070).

Binimetinib, a MEK inhibitor approved for treatment of *BRAF* (*V600E*) mutant tumors in patients with unresectable or metastatic melanoma, was explored for use in patients with recurrent or persistent LGSOC (Table 1: NCT01849874). Binimetinib treatment did not improve PFS at the time of preliminary analysis, leading to early closure of the phase II study. However, post-hoc analysis revealed that patients with *KRAS* mutations were 3.4 times more likely to respond to binimetinib than patients without *KRAS* mutations.[90] Currently, binimetinib is included in the National Cancer Network compendium for treatment of recurrent LGSOC.

Binimetinib in combination with bulparlisib (a PI3K inhibitor) was examined in a clinical trial conducted in patients with advanced solid tumors including RAS/BRAF mutant ovarian carcinomas (Table 1: NCT01363232). Although the

dual inhibition showed promising activity with 12% patients with RAS/BRAF mutant ovarian cancer showing a partial response, the continuous dosing regimen led to intolerable toxicity.[91] Consequently, alternative dosing strategies when combining therapies must be explored to achieve maximum benefit.

Pimasertib, an allosteric MEK inhibitor, has been shown to possess efficacy in combination with voxtalisib (a PI3K inhibitor) in 65 patients with unresectable LGSOC, peritoneal carcinoma, or serous borderline ovarian tumors.[92] Although monotherapy resulted in slightly better PFS and ORR than combination therapy, encouragingly, stable disease was achieved in 50% of patients in the combination group and 40% of patients in the pimasertib monotherapy group (Table 1: NCT01936363). The study was discontinued early because of the lack of ORR suggesting further research is needed to study the utility of combined MEK and PI3K inhibition.

A dual RAF/MEK covalent inhibitor called VS-6766 is currently in a phase II clinical trial to compare the efficacy of monotherapy to combination with defactinib, a focal adhesion kinase (FAK) inhibitor, in recurrent LGSOC (Table 1: NCT04625270). The most recent update of the clinical study reported the combination elicited an ORR of 70% in patients with KRAS-mutant LGSOC and 44% in those with KRAS wild-type. Based on the promising results, the FDA has granted a breakthrough therapy designation to the combination, irrespective of *KRAS* mutational status.

Perspective and Future Directions

Promising pre-clinical and clinical studies have demonstrated the potential for targeting the RAS/MAPK in EOC subtypes. Combination therapy with novel agents targeting RAF and MEK may benefit patients with type I ovarian cancers and should be investigated. Patients that received combination therapy with RAF and MEK inhibitors derived promising early clinical results demanding future research into new combinations for ovarian cancer. Although combination therapy has demonstrated benefits, most patients develop resistance to RAF and MEK inhibitors concomitant with disease progression in less than a year.[93]

Unfortunately, in response to MAPK pathway inhibition, compensatory mechanisms are activated that cause resistance by activating feedback loops in tumor cells.[94] A clinical study treating metastatic melanoma harboring *BRAF* mutation found combined BRAF and MEK inhibition resulted in significant reduction of disease progression. The results suggested that 25% decreased risk of resistance could be achieved by inhibition of the MAPK pathway at two nodes rather than one.[95] In addition, several clinical trials have investigated the efficacy of ERK inhibitors in treating advanced staged malignancy which displays resistance to targeting upstream nodes of the MAPK pathway (Table 1: NCT04055649, NCT01781429, and NCT02711345). Future studies will establish the clinical efficacy of pharmacologic inhibition of ERK in combination with other MAPK inhibitors to promote increased tumor sensitivity.

While standard chemotherapy with carboplatin and paclitaxel achieves an improved clinical response, resistance to chemotherapy is a major impediment in the management of ovarian cancer.[2] In the clinic it has been observed that patients with type I ovarian cancers harboring gain-of-function MAPK activation respond poorly to platinum-based chemotherapy.[26] Early studies have demonstrated the combination of EGFR, BRAF, or MEK inhibitors with standard chemotherapy agents significantly improves clinical efficacy and delays drug resistance in platinum refractory cancer.[26] The combined treatment of trametinib, an allosteric MEK1/2 inhibitor and selumetinib have identified *KRAS* mutational status, EGFR and PKC-alpha protein expression as predictive biomarkers that distinguish MEK inhibitor sensitive and MEK resistant LGSOC cell lines.[96] The data suggest that combination therapy of MEK inhibition with EGFR inhibitors may represent a promising new therapy for MEK-resistant LGSOC.

Clinical studies have demonstrated the efficacy of both trametinib and selumetinib monotherapy with promise in LGSOC over cytotoxic chemotherapy. Trametinib may be more potent compared with selumetinib with efficacy correlated to degree of inhibition of ERK phosphorylation.[96] Mechanistically, trametinib shows equal potency for targeting MEK1 and MEK2 and preferentially binds unphosphorylated MEK preventing RAF-dependent MEK activation.[97] In contrast, selumetinib does not block MEK binding and phosphorylation by RAF. With demonstrated occurrence of gain-of-function MAPK mutation in multiple subtypes of EOC, future clinical studies should address the efficacy of MEK inhibition as a general treatment option.

PARP inhibitors have valuable roles as maintenance therapy in EOC; however, like platinum chemotherapy, most patients acquire resistance.[97] Clinical trials in EOC testing combinations of MAPK pathway inhibitors in combination with PARP

inhibitors have presented with limited success. Promisingly, an in vitro study demonstrated the combined treatment with talazoparib (PARP inhibitor) and trametinib (MEK inhibitor) evoked synergistic cytotoxic effects in ovarian cancer cell lines. Further, MEK inhibition by trametinib was capable of desensitizing talazoparib-resistant cells.[98] Another study which combined olaparib (PARP inhibitor) with the pimasertib (MEK inhibitor) demonstrated that pimasertib treatment enhanced PARP inhibition sensitivity in *BRCA2*-proficient ovarian cancer cell lines.[99] These results suggest the utility of MAPK pathway inhibition in combination with PARP inhibitors may offer unexplored therapeutic potential.

Novel approaches to target RAS-driven cancers, including ovarian carcinomas, must be developed and take into consideration isoform and codon-specific mutations in RAS genes observed in tumors. Currently, enhanced efforts are being applied to develop specific inhibitors of all RAS isoforms and mutant RAS alleles that will eventually provide an approach for all RAS-driven cancers including EOC.[8] Due to lack of RAS mutant-specific inhibitors, other strategies, such as RNA interference (RNAi) using small interfering RNAs (siRNA) or antisense oligonucleotides (ASO) to target mutant RAS mRNA are emerging to inhibit RAS-driven cancer.[100] Research efforts are currently focused on modifications and delivery platforms to improve activity, stability, and biosafety.[101] An ongoing phase I trial is attempting to establish preliminary efficacy of exosomes loaded with *KRAS* (*G12D*) siRNA for treating metastatic pancreatic cancer (Table 1: NCT03608631). If successful, siRNA therapies may eventually be extended to treat ovarian cancer with known RAS mutations.

Currently, there is a critical need to identify biomarkers as effective predictors for ovarian cancer diagnosis and prognosis.[20] Biomarkers will increase the capacity to predict therapeutic efficacy and response to treatment, and potentially could enable improvements in early diagnosis and survival of patients with ovarian cancer.[102] A phase II clinical study has been initiated to evaluate biomarker-driven therapies in patients with persistent or recurrent EOC (Table 1: NCT04931342). The study will evaluate the efficacy and safety profile of multiple biomarker-selected treatments with one experimental arm designed to treat patients with cobimetinib whose tumors harbor activating mutations in *KRAS, NRAS, BRAF,* and *NF1*. The study has the potential to detect biomarkers that predict response of cancers to RAS/MAPK targeted therapies.

Conclusion

In summary, the RAS/MAPK pathway has been implicated in ovarian cancer cell survival, tumorigenesis, invasiveness, angiogenesis, and platinum resistance.[10,103,104] The numerous studies discussed herein suggest oncogenic mutations that promote gain-of-function MAPK signaling are prognostic markers and promising therapeutic targets in all subtypes of ovarian cancer. Indeed, targeting the RAS/MAPK pathway in the clinical setting is an emerging tractable strategy for personalized therapy and improved prognosis for woman with ovarian cancer.

Clinical Data Collection

The search strategy for identifying clinical studies included keywords chosen to perform a systematic search of ClinicalTrials.gov database (https://www.clinicaltrials.gov/). Keywords and terms for finding clinical studies included ovarian cancer, type I, type II epithelial ovarian cancer, high and low grade serous ovarian carcinoma, clear cell carcinoma, mucinous carcinoma, endometrioid carcinoma, MAPK, RAS, RAF, MEK, ERK, HER2, ERBB2, and receptor tyrosine kinase.

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