Kinome directed target discovery and validation in unique ovarian clear cell carcinoma models

Caumanns, Joost

Citation for published version (APA):
Caumanns, J. (2019). Kinome directed target discovery and validation in unique ovarian clear cell carcinoma models. [Groningen]: University of Groningen.

CHAPTER 7

Summary and discussion

SUMMARY

Ovarian clear cell carcinoma (OCCC) is the second most common subtype of epithelial ovarian cancer (EOC). EOC has historically been considered one entity, and therefore all subtypes are uniformly treated with optimal cytoreductive surgery and platinum-based chemotherapy (1, 2). Advanced stage diagnosed (FIGO III/IV) OCCC patients have a worse survival compared to stage matched high-grade serous ovarian carcinoma, which is explained by low response rates towards platinum-based chemotherapy (3-8). Efforts to improve OCCC chemotherapy responses have focused on combining platinum with other chemotherapeutic agents or targeted therapies, but have unfortunately not led to higher survival rates (9-11). Accordingly, there is an urgent need to identify novel therapeutic targets and chemotherapy combinations to improve survival of OCCC patients. SWI/SNF chromatin remodeling complexes are important regulators of chromatin structure and gene transcription. Multiple SWI/SNF subunits are genetically altered in cancer. The SWI/SNF DNA targeting subunit *ARID1A* is frequently mutated with the highest mutation frequency found in OCCC (12, 13).

The research presented in this thesis aimed to identify new therapeutic targets for the treatment of OCCC. To this end, we searched for specific kinase vulnerabilities in OCCC with and without deleterious mutations in *ARID1A*.

The high prevalence of *ARID1A* deleterious mutations in OCCC (40-57%) provides an excellent opportunity for synthetic lethal approaches in this ovarian cancer subtype. Synthetic lethality describes a relation between two genes where cells are still viable after loss of one gene but a lethal phenotype occurs after artificial loss of both genes. In **chapter 2**, we reviewed recent studies that performed synthetic lethality screens in an *ARID1A* mutant background in OCCC and other cancers. Advantages and drawbacks of these studies and the clinical relevance of the identified targets were discussed. We focused on synthetic lethal strategies in *ARID1A* mutant OCCC and, in addition, evaluated targets with synthetic lethal effects in other *ARID1A* mutant cancers for their applicability to OCCC. Inhibition of the epigenetic regulators EZH2, HDAC2, HDAC6 and BRD2 was found to be specifically lethal in *ARID1A* mutant OCCC and may be exploited clinically. The DNA repair proteins PARP and ATR were verified as lethal hits in other *ARID1A* mutant cancers and drugs targeting these proteins are currently being investigated in various clinical trials. However, PARP and ATR remain to be assessed as synthetic lethal targets in *ARID1A* mutant OCCC.

Since *ARID1A* mutations are found in around 50% of OCCCs, we pursued a rational approach to specifically target OCCC cell lines with *ARID1A* mutations. Therefore, in **chapter 3**, shRNA based synthetic lethality screens were performed in a large panel of *ARID1A* wild-type and mutant OCCC cell lines (n=14). Given that over half of the human kinases (kinome) are chemically druggable, we specifically explored kinome-centered lethality screens to maximize the chance to identify therapeutically actionable targets (14). Knockdown of the epigenetic reader BRD2 proved to be predominantly lethal in *ARID1A* mutant OCCC cells. Importantly, small molecule inhibitors of the BET bromodomain protein family, to which BRD2 belongs, specifically inhibited proliferation in *ARID1A* mutant OCCC cell lines, both in vitro and in xenografts, and in patient-derived xenografts (PDX) of OCCC. BET inhibition reduced the expression of ARID1A's mutual exclusive partner ARID1B and other SWI/SNF subunits,

presenting causal evidence for the observed lethal interaction with ARID1A mutated OCCC. Our data indicate that BET inhibition may represent a novel treatment strategy for a subset of ARID1A mutated OCCC.

In **chapter 4**, we aimed to identify new kinase mutations and copy number alterations (CNAs) in tumors from a large set of OCCC patients (n=124) and cell lines (n=17) and we subsequently tested the druggability of downstream affected pathways in vitro and in PDX models of OCCC. The human kinome (518 kinases) and additional cancer related genes were sequenced and CNAs were determined by SNP array analysis. Several putative low-frequency driver mutations in kinases not previously annotated in OCCC were identified. The PI3K/AKT/mTOR pathway, MAPK pathway or ERBB family of receptor tyrosine kinases were affected in 91% of all tumors and the DNA repair pathway in 82% of all tumors, as determined from combined mutation and CNA data. Strong p-S6 staining in OCCC patients suggested high activity of mTORC1/2, a key regulator that acts downstream of the PI3K/AKT/mTOR pathway, MAPK pathway and ERBB family of receptor tyrosine kinases. The majority of OCCC cell lines were exceptionally sensitive to mTORC1/2 inhibition by AZD8055 whereas drugs targeting ERBB family of receptor tyrosine kinases or DNA repair signaling had low efficacy. Conforming these findings, we demonstrated efficacy of mTORC1/2 inhibition in our three unique OCCC PDX models. These preclinical data strongly indicate inhibition of mTORC1/2 as an effective treatment strategy, which should be further explored clinically in OCCC.

Sequencing studies by ourselves and other groups presented a heterogeneous mutation pattern in OCCC across PI3K/AKT/mTOR and MAPK proliferation pathways converging into mTORC1/2 activation. Accordingly, in **chapter 5**, we searched for effective combinations of PI3K/AKT/mTOR and MAPK kinase inhibitors in low-dose concentrations to simultaneously target key kinases in OCCC. Small molecule inhibitors of mTORC1/2 (AZD8055), PI3K (GDC0941) and MEK1/2 (selumetinib), were combined at monotherapy IC_{20} doses in a panel of genetically diverse OCCC cell lines (n=7) to determine an optimal low-dose combination. IC_{20} combinations of AZD8055, GDC0941 and selumetinib effectively inhibited proliferation in all seven cell lines. This triple combination reduced kinase activity in PI3K/AKT/mTOR and MAPK pathways, prevented single inhibitor induced feedback mechanisms and inhibited short and long-term proliferation. Furthermore, this low-dose triple drug combination treatment significantly reduced tumor growth in two genetically characterized OCCC patient-derived xenograft (PDX) models without resulting in weight loss in these mice. The effectiveness and tolerability of this combined therapy in PDX models also warrants clinical exploration of this treatment strategy for OCCC.

In chapter 3, 4 and 5 we have used PDX models that may help to improve the predictive value of in vivo testing of novel treatment strategies. These models are thought to better represent patient characteristics compared to cell line based xenografts. In **chapter 6**, we describe the establishment of seven OCCC PDX models and compared histopathology, mutation status, and copy number profiles between paired patient and PDX OCCC tumors to determine the level of similarity. Successful engraftment of OCCC patient tumors was obtained for seven patients (50%). Primary implantation (F1) showed a higher engraftment with fresh patient tumor tissue (five/seven) versus vitrified tumor tissue (two/seven). Success rate of implanted tumor pieces in F2 was higher than those in F1. In addition, latency time was 50% shorter and, in agreement with Ki67 staining results, tumor growth rate was faster in F2. Mutations in the OCCC-related genes *ARID1A*, *PIK3CA*, *PTEN*, *ATM* and *BRCA1* were retained during engraftment. Morphological features and tumor copy number alterations were also comparable between paired tumor and F2 PDXs. Furthermore, several proliferative pathways were enriched both in paired tumors and F2 PDXs. Accordingly, these PDXs may serve as relevant preclinical models for future translational research in OCCC.

DISCUSSION AND FUTURE CONSIDERATIONS

Approaches used for target identification and validation

In this thesis we aimed to discover druggable proteins in OCCC by 1) kinome directed synthetic lethality screening and 2) by kinome sequencing and copy number analysis. The first approach, implemented in chapter 3, was designed to uncover druggable genes that are synthetic lethal with *ARID1A* mutations in OCCC. Accordingly, we screened a library of shRNAs in *ARID1A* mutant versus wild-type OCCC cell lines that specifically target the human kinome. Approximately half of the kinome is chemically druggable and many kinase targeting compounds are in clinical development (14). The enrichment of druggable kinases compared to the amount of druggable genes in the whole genome would increase the probability to find therapeutically actionable targets when specifically screening for kinases. The kinome screening strategy appeared to be successful with the identification of the synthetic lethal and druggable hit BRD2. *BRD2* was a hit in five out of nine *ARID1A* mutant cell lines in our shRNA kinome synthetic lethality screen. Re-validation of two *BRD2* shRNAs showed substantial but not full knockdown of *BRD2* expression (chapter 3, Fig. S3A). Recently, CRISPR-Cas9 knockout screening became available by uncovering a higher number of genes essential for survival across all and subgroups of cancer cell lines tested. CRISPR-Cas9 based screens introduce gene knockouts that assure total abolishment of protein function and are therefore a robust alternative to shRNA synthetic lethality screens, especially for genes where full loss of protein function is required to identify a synthetic lethal hit (15-17). Notably, CRISPR-CAS9 mediated knockout of *BRD2* was lethal in most OCCC cell lines, including *ARID1A* wild-type and mutant cell lines (data not shown). This indicates that, although BRD2 expression level dependency is higher in *ARID1A* mutant OCCC cell lines, some expression is essential for cell survival of both *ARID1A* wild-type and mutant OCCC cells. Downregulation of expression to a minimum activity threshold by shRNAs or chemical inhibition of protein function may therefore be a superior method for identification of synthetic lethal hits, such as BRD2, for which a minimum expression level is essential in all cells. Still, it will be interesting to perform genome-wide CRISPR-Cas9 knockout screening to find additional synthetic lethal hits in *ARID1A* mutant OCCC cell lines and in OCCC cell lines with other frequently mutated genes in OCCC, such as *PIK3CA*, *KRAS* and *TP53*.

The synthetic lethal effect of BRD2 inhibition in *ARID1A* mutant OCCC cells can be mechanistically explained by the transcriptional regulatory role of BRD2 on *ARID1B* and possibly two other SWI/SNF complex members, as demonstrated with chromatin immunoprecipitation (ChIP) sequencing (18). These findings follow a previous report, which demonstrated that *ARID1B* is essential for survival of *ARID1A* mutant cells (19). However, because BRD2 is a broad transcriptional regulator, it is possible that it regulates the transcription of additional essential factors in an *ARID1A* mutant context. These factors can be explored by a genome-wide comparison of already available BRD2 ChIP-sequencing data of *ARID1A* mutant (HAC2) versus wild-type (OVCA429) OCCC cells and more importantly in future ChIP-sequencing experiments using isogenic *ARID1A* mutant cell line pairs.

In the second approach to discover druggable genes in OCCC, as described in chapter 4, we performed sequencing and copy number analysis of the kinome and determined novel significantly mutated kinases and kinase regulatory components in OCCC including *AKT1*, *PIK3R1*, *ERBB3* and *ATM*. These novel mutations and CNAs in combination with other re-validated high and low-frequency alterations in OCCC led us to screen for vulnerabilities towards inhibitors of the PI3K/AKT/mTOR pathway, DNA repair pathway and ERBB family of receptor tyrosine kinases which revealed abundant mTORC1/2 inhibition sensitivity. By specifically sequencing kinases and kinase regulatory components we obtained accurate sequencing results (i.e. high read coverage) and simultaneously increased the possibility to find chemically druggable targets.

SNP array analysis identified 324 significantly amplified genes and 118 significantly deleted genes. During CNAs analysis we focused on significantly amplified and deleted kinases and other cancer related genes included in kinome sequencing. This list consisted of 12 amplified kinases (3.7%) and 5 deleted kinases (4.2%), leaving a large number of copy number altered genes to be further studied. For example, the most significant amplified region 2q14.2 contains the zinc finger transcription factor *GLI2* and is amplified in 40 OCCC tumors (37%). GLI2 is described to act as an oncogenic transcription factor activated downstream of sonic hedgehog signaling and the TGF-β and SMAD family and could be a prominent target in OCCC (20). Besides, GLI2 can be activated non-canonically via the PI3K/AKT/mTOR pathway (21). It is promising that the GLI2 targeting agent GANT61 recently demonstrated in vitro and in vivo efficacy in colorectal cancer (22).

Our data set can be expanded by whole-genome sequencing that is becoming an affordable method and equally robust to targeted sequencing. This may reveal additional OCCC mutations and signatures of nucleotide substitutions in OCCC (23). In contrast to targeted sequencing, whole-genome sequencing can uncover tumor mutational load and thereby predict the frequency of neoepitopes and putative responsiveness of OCCC towards immune checkpoint therapy (24). If sufficient tumor tissue is available, analysis of mRNA expression and the proteome will add valuable information to sequencing and copy number analysis of the OCCC genome-wide. The downstream effects of copy number gains or losses and specific mutations will be uncovered by mRNA expression. Furthermore, a phosphorylation specific reverse phase protein array may point out kinases that are truly over-activated in OCCC and will allow a more powerful prediction of which genetically deregulated kinases are significant targets in OCCC.

We aimed to effectively target OCCC by low-dose inhibitor combinations, as described in chapter 5. In this respect, three inhibitors targeting PI3K/AKT/mTOR pathway nodes (the mTORC1/2 inhibitor AZD8055 and PI3K inhibitor GDC0941 and) and a MAPK pathway node (the MEK1/2 inhibitor selumetinib) were selected to find combinations that effectively repress proliferation in OCCC cells irrespective of mutation status. The mTORC1/2, PI3K and MEK1/2 inhibitors were selected because mutations and CNAs in *PIK3CA*, *PIK3R1*, *AKT*, *KRAS*, *NRAS* and *BRAF* were ubiquitously found in OCCC. Both pathways promote mTORC1/2 activation and can cross-activate each other, which provided a rational to find synergistic effects of combined suboptimal inhibition of these signaling pathway nodes (25). An alternative and unbiased strategy would be to screen inhibitor libraries in combination with AZD8055, the compound with high efficacy in all OCCC cell lines. Such a ‘chemical synthetic lethality’ screen could identify unanticipated synergistic effects of inhibitor combinations with AZD8055 and reveal druggable pathway interactions in OCCC. Small molecule inhibitor libraries have been successfully used on cancer cell lines, but are expensive and require careful titration and robotic plate handling (26).

In chapter 6 we established and characterized seven OCCC PDX models. Three PDX models (an *ARID1A* mutant, a *PIK3CA* mutant and a *PIK3CA* and *ARID1A* wild-type PDX), that reflect the most frequent mutations in OCCC, were used for preclinical drug testing along chapters 3, 4 and 5. Still, expansion of our PDX panel will be crucial to obtain a better coverage of the broad spectrum of mutations and CNAs in OCCC. To that end, it will be important to freshly implant OCCC patient tumors given that fresh implantation provides higher take rates compared to implantation from vitrified tumors. Evaluation of treatment efficacy in OCCC PDX models is time consuming and costly and should therefore be considered as a final step in preclinical testing. OCCC primary cultures and organoids could bridge the gap between cell line based analysis and in vivo analysis in OCCC PDX models. Compared to cancer cell lines, tumor primary cultures and organoids are thought to more closely resemble the patient tumor and they can faster be implemented in drug screens compared to PDXs (27, 28). High-grade serous ovarian carcinoma (HGSOC) organoids have been established from primary cultures (29). However, organoids of OCCC are unfortunately lacking and OCCC primary cultures have only been described in small numbers. The establishment of OCCC organoids from PDX models of OCCC could be an alternative approach, but will most likely require different growth conditions (growth factors) compared to HGSOC. These differences underscore the importance to invest in research to establish specific protocols for OCCC primary cultures and organoids, besides OCCC PDX models, that can be used for preclinical evaluation of drugs (30, 31).

Challenges to improve mechanistic understanding and treatment of OCCC

Mutations in *ARID1A* are mutual exclusive with *TP53* mutations in OCCC, as shown by us and others (32, 33). The *TP53* mutant OCCC tumors were enriched for high FIGO stage, suggesting that mutations in this gene most likely are not early onset alterations in the development of OCCC. Surprisingly, nine of the 13 *TP53* mutant tumors did not have additional mutations in the genes we had analyzed (chapter 4, Fig. 3). HGSOC, besides being *TP53* mutant, generally has a low percentage of mutations. Even though the morphology of these *TP53* mutant-*ARID1A* wild-type OCCC tumors was not associated with HGSOC, it can be of interest to investigate if these nine tumors are a subclass of OCCC that approximates HGSOC. Alternatively, the high percentage of *TP53* wild-type tumors (80-95%) provides an opportunity to re-activate p53 protein in *TP53* wild-type OCCC, ultimately resulting in p53-mediated apoptosis. An extensively studied approach to induce p53 activity is by preventing the interaction of MDM2 with p53, thereby preventing proteasomal degradation of p53 via MDM2 (34). Inhibitors of the MDM2-p53 interaction, such as nutlin-3a and more recently idasanutlin (RG7388), showed synergistic activity in combination with cisplatin in *TP53* wild-type ovarian cancer cell lines (OCCC was not included) (35, 36). Nutlin-3a upregulated p53 levels in OCCC but the combination with cisplatin remains to be tested (37). In a study by Bitler *et*. *al*. p53-mediated apoptosis (through p53-lysine120 acetylation) was specifically induced in *ARID1A* mutant OCCC cells after treatment with the HDAC6 inhibitor ACY1215, as discussed in chapter 2 (30). Future research in OCCC may focus on combining ACY1215 with cisplatin to activate p53 and on combinations of ACY1215 with inhibitors of the MDM2-p53 interaction. Additionally, targeting of other DNA repair genes is of interest in light of our data as presented in chapter 4, in which we described mutations and CNAs in DNA repair proteins in 82% of OCCC tumors. Although DNA repair alterations (including *BRCA1* mutations) were also prominent in OCCC cell lines, low efficacy of the PARP1/2 inhibitor olaparib was observed in these cell lines (n=17). Another study identified efficacy of the PARP trapping agent talazoparib in OCCC cell lines (38). Here, OCCC cell lines with a low IC_{50} for talazoparib more often lacked homologous recombination (HR) capacity, suggesting a rationale to treat HR deficient OCCC with PARP trapping inhibitors. The frequency of HR deficiency in OCCC, however, is low compared to HGSOC, indicating that only a small subset of OCCC patients may benefit from PARP inhibition (39). The broad spectrum of mutations in the DNA repair pathway in OCCC suggests that other DNA repair proteins (e.g. ATM or ATR) or regulators of the cell cycle (e.g. CHECK1/2 or WEE1 for *TP53* mutant OCCC) are putative targets to respectively abolish DNA repair or force detrimental mitosis in the presence of DNA damage.

The pervasive overexpression of *HNF1β*, a transcription factor that promotes glycogen metabolism, aerobic glycolysis and lactate production, is frequently found in OCCC (40, 41). *HNF1β* overexpressing OCCC cells highly express genes typically involved in the Warburg effect, such as *HK1* and *LDHA* (42). Although the exact mechanisms through which *HNF1β* stimulates these processes remain elusive, the therapeutic targeted. Till now, only a limited number of studies have investigated HNF1β as a therapeutic druggability has been evaluated in OCCC. Buthionine sulphoxamine, an inhibitor that acts downstream of HNF1β, re-sensitized ES2 cells to carboplatin (43). Another study found HNF1β to regulate transcription of the Na^+/K^+-ATPase modulating subunit FXYD2. Digoxin and digitoxin, two cardiac glycosides that inhibit Na^+/K^+-ATPase activity, had therapeutic efficacy in TOV21G cells in vitro and in vivo (44). Altogether, these studies indirectly support the druggability of HNF1β. Direct targeting of HNF1β has been described using calcineurin inhibitors but requires evaluation in OCCC models (45).

In chapter 3, we showed that inhibition of the BET bromodomain protein BRD2 is synthetic lethal with *ARID1A* mutations in OCCC. Other established *ARID1A* mutant OCCC synthetic lethal targets are the epigenetic regulators EZH2, HDAC2 and HDAC6 and the SRC family protein YES1 that were discussed in chapter 2. Combined targeting of these proteins, known to be synthetic lethal in *ARID1A* mutant OCCC, can be used to further enhance efficacy. Simultaneous inhibition of these targets at suboptimal dose, in a large panel of OCCC cell lines to resemble the heterogeneous spectrum of mutations in OCCC, similar to the approach described in chapter 5, might be useful to generate synergistic lethality in *ARID1A* mutant OCCC and concurrently prevent systemic toxicity.

OCCC shares a number of

pathological characteristics and genomic alterations with clear cell renal cell carcinoma (CCRC) and endometrial clear cell carcinoma (ECCC), albeit mutation frequencies vary. *TP53* mutations are found at a lower frequency in CCRC (2.2%) and at a higher frequency in ECCC (46%) compared to OCCC (11%) (46, 47). In all three cancer subtypes the majority of tumors have high *HNF1β* expression (48, 49). *ARID1A* mutations are less frequently found in CCRC (4.6%) and ECCC (21%) compared to 46% in OCCC (47, 50). Moreover, overlap with OCCC PI3K/AKT/mTOR pathway mutations is found in CCRC (*PTEN*, 11%) and ECCC (*PIK3CA*, 36%; *FBXW7*, 25% and *PIK3R1*, 18%) (47, 50). The transcription factor *GLI2*, strongly amplified in OCCC, is also frequently overexpressed in CCRC. High expression levels of GLI2 correlated with worse overall survival in CCRC patients, which may guide studies in OCCC patients (21). Considering these commonalities, future research in OCCC could take advantage from studies performed in CCRC and ECCC.

Improvements in therapy options for OCCC patients

The results presented in chapter 3, 4 and 5 aim towards clinical evaluation of BET bromodomain inhibition, mTORC1/2 inhibition and combined low-dose mTORC1/2, PI3K and MEK1/2 inhibition in OCCC, respectively.

BET bromodomain inhibition is extensively being studied in the clinic. There are 17 compounds in ongoing trials (chapter 2, Table 1) from which iBET-762 (GSK525762) is currently tested in a phase II combination trial with fulvestrant in ER^+ breast cancer (NCT02964507). BET bromodomain inhibition and the ER degrader fulvestrant acted synergistic in preclinical ER^+ breast cancer models (51). Intermediate results from a phase I/II trial with iBET-762 in acute myeloid leukemia described two dose limiting toxicities on a total of 46 patients. The authors conclude that iBET-762 treatment related adverse events in AML subjects were manageable and reversible (52). These preliminary clinical data further support the evaluation of BRD2 inhibition by iBET-762 in *ARID1A* mutant OCCC patients in a future phase II trial.

Treatment with the mTORC1/2 inhibitors AZD8055 and OSI-027 provided anti-tumor efficacy only above maximum tolerated dose, resulting in discontinuation of these two drugs in patients (53, 54). Phase II evaluation of MLN0128 (sapanisertib), a novel mTORC1/2 inhibitor, is ongoing in CCRC and endometrial cancer (NCT02724020 and NCT02725268). Efficacy determination of MLN0128 alone remains to be performed in OCCC patients. Interestingly, a new phase II trial combining MLN0128 with standard of care paclitaxel is scheduled in epithelial ovarian cancer, including all subtypes (NCT03648489). Probably, some OCCC patients will be included, which may demonstrate the added value of MLN0128 combined with paclitaxel in this ovarian cancer subtype.

A low-dose combination of mTORC1/2, PI3K and MEK1/2 inhibitors could be assessed with MLN0128 and new generation PI3K and MEK1/2 inhibitors. No clinical trials have been performed combining three kinase inhibitors. Accordingly, a careful dose-escalation of mTORC1/2, PI3K and MEK1/2 inhibitors in OCCC patients will be crucial to find maximum efficacy of this strategy while minimizing systemic toxicity.

The molecular distinction between OCCC and other ovarian cancer subtypes and the genetic heterogeneity between OCCC patients, as demonstrated in this thesis, indicate that future targeted therapy clinical trials in ovarian cancer should be subtype specific. Given the infrequency of OCCC multicenter (international) trials will be necessary to obtain adequate numbers of patients in OCCC directed clinical trials. Currently ongoing multicenter trials that focus on OCCC are directed against the immune modulatory receptors TIM1 (NCT02837991), PD-1 (NCT03355976) and CTLA4 and PD-L1 combined with chemotherapy (NCT03405454). For clinical evaluation of BRD2 inhibition in *ARID1A* mutant OCCC, a basket trial can be performed in order to reach sufficient patient numbers. In this approach *ARID1A* mutant OCCC would be included together with *ARID1A* mutant tumors from a different origin (for example *ARID1A* mutant CCRC and ECCC). However, the effectiveness of targeting BRD2 in CCRC and ECCC first needs to be proven preclinically.

CONCLUSION

In this thesis, new therapeutic targets in OCCC have been identified and a low-dose treatment strategy was preclinically tested in unique OCCC models. These results may advance the treatment of OCCC.

REFERENCES

1. Oliver KE, Brady WE, Birrer M, Gershenson DM, Fleming G, Copeland LJ, et al. An evaluation of progression free survival and overall survival of ovarian cancer patients with clear cell carcinoma versus serous carcinoma treated with platinum therapy: An NRG Oncology/Gynecologic Oncology Group experience. Gynecol Oncol. 2017 Nov;147(2):243-9.
2. Vaughan S, Coward JI, Bast RC,Jr, Berchuck A, Berek JS, Brenton JD, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer. 2011 Sep 23;11(10):719-25.
3. Anglesio MS, Carey MS, Kobel M, Mackay H, Huntsman DG, Vancouver Ovarian Clear Cell Symposium Speakers. Clear cell carcinoma of the ovary: a report from the first Ovarian Clear Cell Symposium, June 24th, 2010. Gynecol Oncol. 2011 May 1;121(2):407-15.
4. Sugiyama T, Kamura T, Kigawa J, Terakawa N, Kikuchi Y, Kita T, et al. Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. Cancer. 2000 Jun 1;88(11):2584-9.
5. Takano M, Kikuchi Y, Yaegashi N, Kuzuya K, Ueki M, Tsuda H, et al. Clear cell carcinoma of the ovary: a retrospective multicentre experience of 254 patients with complete surgical staging. Br J Cancer. 2006 May 22;94(10):1369-74.
6. Ho CM, Huang YJ, Chen TC, Huang SH, Liu FS, Chang Chien CC, et al. Pure-type clear cell carcinoma of the ovary as a distinct histological type and improved survival in patients treated with paclitaxel-platinum-based chemotherapy in pure-type advanced disease. Gynecol Oncol. 2004 Jul;94(1):197-203.
7. Pectasides D, Fountzilas G, Aravantinos G, Kalofonos C, Efstathiou H, Farmakis D, et al. Advanced stage clear-cell epithelial ovarian cancer: the Hellenic Cooperative Oncology Group experience. Gynecol Oncol. 2006 Aug;102(2):285-91.
8. Utsunomiya H, Akahira J, Tanno S, Moriya T, Toyoshima M, Niikura H, et al. Paclitaxel-platinum combination chemotherapy for advanced or recurrent ovarian clear cell adenocarcinoma: a multicenter trial. Int J Gynecol Cancer. 2006 Jan-Feb;16(1):52-6.
9. Takakura S, Takano M, Takahashi F, Saito T, Aoki D, Inaba N, et al. Randomized phase II trial of paclitaxel plus carboplatin therapy versus irinotecan plus cisplatin therapy as first-line chemotherapy for clear cell adenocarcinoma of the ovary: a JGOG study. Int J Gynecol Cancer. 2010 Feb;20(2):240-7.
10. Sugiyama T, Okamoto A, Enomoto T, Hamano T, Aotani E, Terao Y, et al. Randomized Phase III Trial of Irinotecan Plus Cisplatin Compared With Paclitaxel Plus Carboplatin As First-Line Chemotherapy for Ovarian Clear Cell Carcinoma: JGOG3017/GCIG Trial. J Clin Oncol. 2016 Aug 20;34(24):2881-7.
11. Farley JH, Brady WE, Fujiwara K, Nomura H, Yunokawa M, Tokunaga H, et al. A phase II evaluation

of temsirolimus in combination with carboplatin and paclitaxel followed by temsirolimus consolidation as first-line therapy in the treatment of stage III-IV clear cell carcinoma of the ovary. JCO. 2016 05/20; 2017/11;34(15):5531-.

12. Jones S, Wang TL, Shih I, Mao TL, Nakayama K, Roden R, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010 Oct 8;330(6001):228-31.
13. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010 Oct 14;363(16):1532-43.
14. Hu Y, Furtmann N, Bajorath J. Current compound coverage of the kinome. J Med Chem. 2015 Jan 8;58(1):30-40.
15. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelson T, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science. 2014 Jan 3;343(6166):84-7.
16. Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR-Cas9 system. Science. 2014 Jan 3;343(6166):80-4.
17. Hart T, Chandrashekhar M, Aregger M, Steinhart Z, Brown KR, MacLeod G, et al. High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities. Cell. 2015 Dec 3;163(6):1515-26.
18. Berns K, Caumanns JJ, Hijmans EM, Gennissen AMC, Severson TM, Evers B, et al. ARID1A mutation sensitizes most ovarian clear cell carcinomas to BET inhibitors. Oncogene. 2018 May 15.
19. Helming KC, Wang X, Wilson BG, Vazquez F, Haswell JR, Manchester HE, et al. ARID1B is a specific vulnerability in ARID1A-mutant cancers. Nat Med. 2014 Mar;20(3):251-4.
20. Javelaud D, Alexaki VI, Dennler S, Mohammad KS, Guise TA, Mauviel A. TGF-beta/SMAD/GLI2 signaling axis in cancer progression and metastasis. Cancer Res. 2011 Sep 1;71(17):5606-10.
21. Zhou J, Zhu G, Huang J, Li L, Du Y, Gao Y, et al. Non-canonical GLI1/2 activation by PI3K/AKT signaling in renal cell carcinoma: A novel potential therapeutic target. Cancer Lett. 2016 Jan 28;370(2):313-23.
22. Tang YA, Chen YF, Bao Y, Mahara S, Yatim SMJM, Oguz G, et al. Hypoxic tumor microenvironment activates GLI2 via HIF-1alpha and TGF-beta2 to promote chemoresistance in colorectal cancer. Proc Natl Acad Sci U S A. 2018 Jun 26;115(26):E5990-9.
23. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013 Aug 22;500(7463):415-21.
24. Shen J, Ju Z, Zhao W, Wang L, Peng Y, Ge Z, et al. ARID1A deficiency promotes mutability and potentiates therapeutic antitumor immunity unleashed by immune checkpoint blockade. Nat Med. 2018 May 7.
25. Rozengurt E, Soares HP, Sinnet-Smith J. Suppression of feedback loops mediated by PI3K/mTOR induces multiple overactivation of compensatory pathways: an unintended consequence leading to drug resistance. Mol Cancer Ther. 2014 Nov;13(11):2477-88.
26. Lampis A, Carotenuto P, Vlachogiannis G, Cascione L, Hedayat S, Burke R, et al. MIR21 Drives Resistance to Heat Shock Protein 90 Inhibition in Cholangiocarcinoma. Gastroenterology. 2018 Mar;154(4):1066,1079.e5.
27. Miserocchi G, Mercatali L, Liverani C, De Vita A, Spadazzi C, Pieri F, et al. Management and potentialities of primary cancer cultures in preclinical and translational studies. J Transl Med. 2017 Nov 7;15(1):229,017-1328-z.
28. Drost J, Clevers H. Organoids in cancer research. Nat Rev Cancer. 2018 Jul;18(7):407-18.
29. Jabs J, Zickgraf FM, Park J, Wagner S, Jiang X, Jechow K, et al. Screening drug effects in patient-derived cancer cells links organoid responses to genome alterations. Mol Syst Biol. 2017 Nov 27;13(11):955.
30. Bitler BG, Wu S, Park PH, Hai Y, Aird KM, Wang Y, et al. ARID1A-mutated ovarian cancers depend on HDAC6 activity. Nat Cell Biol. 2017 Aug;19(8):962-73.
31. Fukumoto T, Park PH, Wu S, Fatkhutdinov N, Karakashev S, Nacarelli T, et al. Repurposing Pan-HDAC Inhibitors for ARID1A-Mutated Ovarian Cancer. Cell Rep. 2018 Mar 27;22(13):3393-400.

32. Guan B, Wang TL, Shih I. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. Cancer Res. 2011 Nov 1;71(21):6718-27.
33. Caumanns JJ, Berns K, Wisman GBA, Fehrmann RSN, Tomar T, Klip H, et al. Integrative kinome profiling identifies mTORC1/2 inhibition as treatment strategy in ovarian clear cell carcinoma. Clin Cancer Res. 2018 Apr 23.
34. Ashcroft M, Vousden KH. Regulation of p53 stability. Oncogene. 1999 Dec 13;18(53):7637-43.
35. Meijer A, Kruyt FA, van der Zee AG, Hollema H, Le P, ten Hoor KA, et al. Nutlin-3 preferentially sensitises wild-type p53-expressing cancer cells to DR5-selective TRAIL over rhTRAIL. Br J Cancer. 2013 Nov 12;109(10):2685-95.
36. Zanjirband M, Edmondson RJ, Lunec J. Pre-clinical efficacy and synergistic potential of the MDM2-p53 antagonists, Nutlin-3 and RG7388, as single agents and in combined treatment with cisplatin in ovarian cancer. Oncotarget. 2016 Jun 28;7(26):40115-34.
37. Crane EK, Kwan SY, Izaguirre DI, Tsang YT, Mullany LK, Zu Z, et al. Nutlin-3a: A Potential Therapeutic Opportunity for TP53 Wild-Type Ovarian Carcinomas. PLoS One. 2015 Aug 6;10(8):e0135101.
38. Wilkerson PM, Dedes KJ, Samartzis EP, Dedes I, Lambros MB, Natrajan R, et al. Preclinical evaluation of the PARP inhibitor BMN-673 for the treatment of ovarian clear cell cancer. Oncotarget. 2017 Jan 24;8(4):6057-66.
39. Chao A, Lai CH, Wang TH, Jung SM, Lee YS, Chang WY, et al. Genomic scar signatures associated with homologous recombination deficiency predict adverse clinical outcomes in patients with ovarian clear cell carcinoma. J Mol Med (Berl). 2018 Jun;96(6):527-36.
40. Okamoto T, Mandai M, Matsumura N, Yamaguchi K, Kondoh H, Amano Y, et al. Hepatocyte nuclear factor-1beta (HNF-1beta) promotes glucose uptake and glycolytic activity in ovarian clear cell carcinoma. Mol Carcinog. 2013 Sep 17.
41. Ye S, Yang J, You Y, Cao D, Huang H, Wu M, et al. Clinicopathologic Significance of HNF-1beta, AIRD1A, and PIK3CA Expression in Ovarian Clear Cell Carcinoma: A Tissue Microarray Study of 130 Cases. Medicine (Baltimore). 2016 Mar;95(9):e3003.
42. Amano Y, Mandai M, Yamaguchi K, Matsumura N, Kharma B, Baba T, et al. Metabolic alterations caused by HNF1beta expression in ovarian clear cell carcinoma contribute to cell survival. Oncotarget. 2015 Sep 22;6(28):26002-17.
43. Lopes-Coelho F, Gouveia-Fernandes S, Goncalves LG, Nunes C, Faustino I, Silva F, et al. HNF1beta drives glutathione (GSH) synthesis underlying intrinsic carboplatin resistance of ovarian clear cell carcinoma (OCCC). Tumour Biol. 2015 Oct 31.
44. Hsu IL, Chou CY, Wu YY, Wu JE, Liang CH, Tsai YT, et al. Targeting FXYD2 by cardiac glycosides potently blocks tumor growth in ovarian clear cell carcinoma. Oncotarget. 2016 Sep 27;7(39):62925-38.
45. Faguer S, Esposito L, Casemayou A, Pirson Y, Decramer S, Cartery C, et al. Calcineurin Inhibitors Downregulate HNF-1beta and May Affect the Outcome of HNF1B Patients After Renal Transplantation. Transplantation. 2016 Sep;100(9):1970-8.
46. Brugarolas J. Molecular genetics of clear-cell renal cell carcinoma. J Clin Oncol. 2014 Jun 20;32(18):1968-76.
47. DeLair DF, Burke KA, Selenica P, Lim RS, Scott SN, Middha S, et al. The genetic landscape of endometrial clear cell carcinomas. J Pathol. 2017 Oct;243(2):230-41.
48. Hoang LN, Han G, McConechy M, Lau S, Chow C, Gilks CB, et al. Immunohistochemical characterization of prototypical endometrial clear cell carcinoma--diagnostic utility of HNF-1beta and oestrogen receptor. Histopathology. 2014 Mar;64(4):585-96.
49. Ji JX, Wang YK, Cochrane DR, Huntsman DG. Clear cell carcinomas of the ovary and kidney: clarity through genomics. J Pathol. 2018 Apr;244(5):550-64.
50. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013 Jul 4;499(7456):43-9.
51. Feng Q, Zhang Z, Shea MJ, Creighton CJ, Coarfa C, Hilsenbeck SG, et al. An epigenomic approach to therapy for tamoxifen-resistant breast cancer. Cell Res. 2014 Jul;24(7):809-19.

52. Dawson M, Stein EM, Huntly BJ, Karadimitris A, Kamdar M, Fernandez de Larrea C, et al. A Phase I Study of GSK525762, a Selective Bromodomain (BRD) and Extra Terminal Protein (BET) Inhibitor: Results from Part 1 of Phase I/II Open Label Single Agent Study in Patients with Acute Myeloid Leukemia (AML). 2017;130(Suppl 1)(1377).
53. Naing A, Aghajanian C, Raymond E, Olmos D, Schwartz G, Oelmann E, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics of AZD8055 in advanced solid tumours and lymphoma. Br J Cancer. 2012 Sep 25;107(7):1093-9.
54. Mateo J, Olmos D, Dumez H, Poondru S, Samberg NL, Barr S, et al. A first in man, dose-finding study of the mTORC1/mTORC2 inhibitor OSI-027 in patients with advanced solid malignancies. Br J Cancer. 2016 Apr 12;114(8):889-96.