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High throughput screening identifies dasatinib as synergistic with trametinib in low grade serous ovarian carcinoma


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HIGHLIGHTS

- We performed a high throughput screen of 1610 compounds across six low grade serous ovarian carcinoma cell lines
- Of 16 prioritised screen hits, 11 compounds passed dose-response validation using cell viability assays
- The most promising hits underwent synergy profiling with the trametinib (dasatinib, disulfram, carfilzomib, romidepsin)
- Disulfram demonstrated excellent selectivity for LGSOC versus high grade serous ovarian carcinoma comparator lines
- Dasatinib demonstrated favourable synergy with the MEK inhibitor trametinib

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ABSTRACT

Background. Low grade serous ovarian carcinoma (LGSOC) is a distinct histotype of ovarian cancer characterised high levels of intrinsic chemoresistance, highlighting the urgent need for new treatments. High throughput screening in clinically-informative cell-based models represents an attractive strategy for identifying candidate treatment options for prioritisation in clinical studies.

Methods. We performed a high throughput drug screen of 1610 agents across a panel of 6 LGSOC cell lines (3 RAS/RAF-mutant, 3 RAS/RAF-wildtype) to identify novel candidate therapeutic approaches. Validation comprised dose-response analysis across 9 LGSOC models and 5 high grade serous comparator lines.

Results. 16 hits of 1610 screened compounds were prioritised for validation based on >50% reduction in nuclei counts in over half of screened cell lines at 1000 nM concentration. 11 compounds passed validation, and the four agents of greatest interest (dasatinib, tyrosine kinase inhibitor; disulfram, aldehyde dehydrogenase inhibitor; carfilzomib, proteasome inhibitor; romidepsin, histone deacetylase inhibitor) underwent synergy profiling with the recently approved MEK inhibitor trametinib. Disulfram demonstrated excellent selectivity for LGSOC versus high grade serous ovarian carcinoma comparator lines (P = 0.003 for IC50 comparison), while the tyrosine kinase inhibitor dasatinib demonstrated favourable synergy with trametinib across multiple LGSOC models (maximum zero interaction potency synergy score 46.9). The novel, highly selective Src family kinase (SKF) inhibitor NXP900 demonstrated a similar trametinib synergy profile to dasatinib, suggesting that SKF inhibition is the likely driver of synergy.

Conclusion. Dasatinib and other SKF inhibitors represent novel candidate treatments for LGSOC and demonstrate synergy with trametinib. Disulfram represents an additional treatment strategy worthy of investigation.

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1. Introduction

Low grade serous ovarian carcinoma (LGSOC) is a unique and uncommon ovarian cancer type characterised by younger patient age and high levels of intrinsic chemoresistance compared to more common high grade serous ovarian carcinomas (HGSOC) [1]. The overall survival time for LGSOC patients is around 10 years from diagnosis [2]. While this is longer than many other ovarian cancer histotypes [3] - including HGSOC - this is largely due to prolonged post-relapse survival, and quality of life during this time is significantly impacted by incrementally advancing disease and accumulating toxicity [1].

The objective response rate of LGSOC to first-line platinum-based chemotherapy regimens is ≤25% [1,4,5], and the response rate to common cytotoxic regimens in the recurrent setting is ≤15% [6]. Treatment of advanced, progressive and recurrent disease therefore represents a major clinical challenge [6]. Poor efficacy of cytotoxic chemotherapy has resulted in a growing interest in molecularly-targeted therapeutics for LGSOC management [1]. The most common molecular features of LGSOC include frequent mutational activation of the RAS-RAF-MEK-ERK pathway, with 33%, 10% and 10% of patients demonstrating KRAS, NRAS and BRAF mutations (RAS/RAF-mutant), respectively [7–9]. Genomic perturbations in USP9X, EIF5AX and CDKN2A are also recurrent in LGSOC, but are present at a lower frequency than RAS/RAF pathway mutations [9]. LGSOC are ubiquitously oestrogen receptor (ER) positive, and frequently express progesterone receptor (PR) [10,11]. Together, these molecular features have suggested RAS/RAF pathway inhibition has also demonstrated considerable efficacy [7].

Recently, the MEK inhibitor trametinib was shown to improve progression-free survival in recurrent or persistent LGSOC, with a response rate of 26% [12]. However, it is clear that not all patients benefit from trametinib, and that not all responses are durable. In particular, response rates in patients whose tumours do not harbour KRAS, NRAS and BRAF mutations (RAS/RAF-mutant), respectively [7–9]. Genomic perturbations in USP9X, EIF5AX and CDKN2A are also recurrent in LGSOC, but are present at a lower frequency than RAS/RAF pathway mutations [9]. LGSOC are ubiquitously oestrogen receptor (ER) positive, and frequently express progesterone receptor (PR) [10,11]. Together, these molecular features have suggested RAS/RAF pathway inhibition and endocrine therapies as potential treatment strategies [6].

2. Materials and methods

2.1. LGSOC cell lines

VOA1056, VOA3723, VOA4627, VOA4698, VOA6406, VOA7681 and VOA13738 were provided by the MD Anderson Cancer Centre (table S1); these cell lines were derived from LGSOC patients as previously reported [17,18]. HOC7 cells [19] were provided by Dr. Carey from the University of British Columbia (table S1); these cells lines were derived from LGSOC patients as previously reported [17,18]. HOC7 cells [19] were provided by the MD Anderson Cancer Centre. HO433 was derived from an LGSOC patient treated at the Edinburgh Cancer Centre; ascites drained from the abdomen of a patient with relapsed LGSOC previously treated with platinum-taxane combination therapy was cultured and differentially trypsinised to produce pure malignant epithelial cells. These cells underwent serial passage and were considered to be immortal after >20 passages and repetitive freeze-thaw recovery.

LGSOC cell lines were grown in 1:1 ratio of 105 and 199 medium (Sigma #M6395 and #M5017) supplemented with 15% foetal bovine serum (FBS) (Gibco #10437028) in standard culture conditions (37 °C, 5% CO2). Cells used in the high throughput screen (HOC7, HO433, VOA1056, VOA3723, VOA4698, VOA7681) underwent staining with Hoechst-33,342 (cell nuclei), Concanavalin A (endoplasmic reticulum) and Phalloidin (F-actin) as previously described [20] to characterise cell morphology. Prior to staining, cells were seeded into a 384-well plate and incubated for 4 days. Cells were then fixed in situ by adding an equal volume of 8% formaldehyde for 30 min and subsequently washed twice with phosphate-buffered saline (PBS). Stained cells were imaged at 20× using the ImageXpress Micro Confocal.

2.2. RAS/RAF mutation status in LGSOC cell lines

HOC7 is known to harbour a KRAS G12A mutation (table S1), as previously described [21]. The RAS/RAF mutation status of VOA1056 (RAS/RAF-mutant: NRAS G61R), VOA3723 (RAS/RAF-wildtype), VOA4627 (RAS/RAF-wildtype), VOA4698 (RAS/RAF-wildtype) and VOA6406 (RAS/RAF-mutant: NRAS G61R) were determined by whole exome sequencing in previous work [17,18]. The mutation status of HO433 (RAS/RAF-wildtype), VOA7681 (RAS/RAF-mutant: KRAS G12V) and VOA13738 (RAS/RAF-wildtype) was determined by Sanger sequencing of regions encompassing KRAS codon 12/13, KRAS codon 61, NRAS codon 12/13, NRAS codon 61 and BRAF codon 600 (supplementary methods 1).

2.3. HGSOC comparator lines

5 HGSOC models from the literature (OVCA3, OVSAHO, OVCAR4, 59M and SNU119) [22] were used as a comparator cell line panel; these models represent TP53-mutant, high confidence HGSOC cell lines lacking genomic events suggestive of other histotypes from consensus analysis [22]. HGSOC cells were grown in RPMI 1640 medium (Gibco #21875034) supplemented with 10% FBS (Gibco #10437028).

2.4. Cell line authentication and mycoplasma testing

All cell lines were authenticated by short tandem repeat profiling prior to experimentation using the Promega GenePrint 10 System (Promega, #B9510). Mycoplasma testing was undertaken every 3–6 months using the Lonza MycoAlert Detection Kit (Lonza #LT07–318) to confirm absence of mycoplasma infection.

2.5. High throughput screen

High throughput screening was performed in 384-well CELLSTAR Cell Culture plates (Greiner #781091) using 6 LGSOC cell lines (3 RAS/RAF-mutant: HOC7, VOA1056, VOA7681; 3 RAS/RAF-wildtype: HO433, VOA3723, VOA4698) (Fig. 1). The drug library comprised 1610 compounds from the Prestwick FDA Approved Chemical Library (1280 agents) and TargetMol Anticancer Compound Library (TargetMol #LT2110, 330 agents). Compound liquid handling steps were performed using a Beckman Automated Workstation (Beckman Coulter).

Cell cultures were trypsinised with trypLE express (Gibco #12605010), counted using the Countess II Automated Cell Counter (Thermofisher Scientific), resuspended in media to the desired concentration, and then seeded into barcoded 384-well plates for screening using a ViaFill reagent dispenser (Integra). Cell seeding densities were optimised for each cell line to achieve approximately 70% confluence at the end of the treatment period in vehicle-treated conditions (range 300–3500 cells per well). Seeded cells were incubated for 24 h prior to drug treatment for 3 days at a single-dose of 1000 nM per...
compound. All wells were normalised to 0.1% DMSO (v/v) with controls (negative: 0.1% DMSO; positive: 1 μM staurosporine). After the treatment period, LGSOC cells were fixed in situ by adding an equal volume of 8% formaldehyde for 30 mins before washing twice with PBS.

2.6. Screen hit identification

Fixed cells underwent staining to identify cell nuclei using Hoechst-33342 (Thermo scientific). Stained cells were imaged at 20× using the ImageXpress Micro Confocal with robotic plate loader (6 sites per well per channel). Images were analysed using MetaXpress built-in analysis modules and the data were normalised and visualised via TIBCO Spotfire Analyst. Total nuclei counts (sum nuclei counts over 6 sites) were normalised to DMSO vehicle control wells on a plate-by-plate basis. Screening QC was carried out (including Z-prime analysis) and screen hits were defined as compounds significantly reducing nuclei count in compound-treated wells versus control wells by >50% (Z-score < −5.5) across more than half of the screened LGSOC cell lines (≥4 of 6 lines).

2.7. Hit validation

Drug screen hits were validated in dose-response studies of resupplied compounds in 96-well cell viability assays. Individual compounds were purchased from Selleckchem for use in validation assays (supplementary methods section 2). NXP900 was supplied by the Unciti-Broceta lab at the CRUK Scotland Centre [23,24]. Seeding densities were optimised for each cell line to achieve approximately 70% confluence in vehicle-treated wells at the end of the experiment. Cells were seeded into CELLSTAR 96-well plates (Greiner #655180), and incubated for 24 h prior to treatment for 5 days using an 8-point 1:3 serial dilutions of test compounds; the start dose was adjusted as necessary for each cell line based on individual sensitivity. Viability of cells treated at each experimental dose were quantified using the Alamar Blue High Sensitivity Assay (supplementary methods section 3) relative to vehicle-treated control wells. Staurosporine treatment (1000 nM concentration) was used as a positive cell death control.

Dose-response data were used to calculate IC50 values, reflecting overall sensitivity of cells to the compound of interest, using the four-
parameter log-logistic function. Compounds with an IC50 < 1000 nM across ≥4 of the 6 models included in the high throughput screen were considered to pass validation.

For validated compounds, dose-response profiling was expanded to a total of 9 LGSOC cell lines (2 KRAS mutant, 2 NRAS mutant, 5 KRAS/ BRAF/NRAS wildtype) (table S1) and 5 HGSOC comparator lines to provide a broader overview of sensitivity ranges and selectivity for LGSOC cells. Presented data are from three independent replicates, with each replicate comprising three technical repeats (N = 9 data points per cell line per compound per dose).

2.8. Combination assays

Leading hits from single-agent studies were taken forward into combination assays to identify potential synergy with trametinib. Four cell lines representing a range of RAS/RAF mutation status and trametinib sensitivity were used for combination assays: HOC7 (RAS/RAF-mutant, highly trametinib sensitive: IC50 < 10 nM), VOA7681 (RAS/RAF-mutant, trametinib sensitive: IC50 < 50 nM), VOA13738 (RAS/RAF-wildtype, trametinib sensitive: IC50 < 50 nM) and HO433 (RAS/RAF-wildtype, trametinib resistant: IC50 > 1000 nM).

Cells were seeded into 96-well plates; 24 h after seeding, cells were treated in a 6 × 6 dose combination matrix for 5 days using 1:3 serial dilutions of trametinib and the drug of interest. Starting doses were optimised per cell line using the single agent dose-response data as a guide. Three plates were seeded and dosed per biological replicate to provide technical triplicates. After 5 days of treatment, viability was determined using the Alamar Blue High Sensitivity Assay (supplementary methods section 3).

2.9. Statistical analyses

All analysis was performed using R 4.2.2 in RStudio 2022.07.2 + 576. Single-agent dose-response data were analysed using the drc R package (version 3.0.1) [25]; IC50 values were calculated using the drm function and four-parameter log-logistic dose response models. Hierarchical clustering of IC50 values was performed using Euclidean distance and Ward’s linkage on a matrix of log2 nM concentrations across cell lines. Combination data were analysed using the SynergyFinder R package (version 3.6.3) [26] to calculate zero interaction potency (ZIP) synergy scores for each combination dose. ZIP score > 10 was used as a threshold to identify likely synergistic effects, as recommended in the SynergyFinder documentation [26].

3. Results

3.1. High throughput screening of LGSOC cell lines

Six cell lines representing both RAS/RAF-mutant (n = 3: HOC7 KRAS G12A, VOA7681 KRAS G12V, VOA1056 NRAS G61R) and RAS/RAF-wildtype (n = 3: HO433, VOA3723, VOA4698; all KRAS, BRAF and NRAS wildtype) (Fig. 1, Fig. 2, table S1) underwent high throughput screening in 384-well format with 1610 compounds from the Prestwick FDA-Approved Compound Library and a TargetMol Anti-Cancer Compound Library (Fig. 1). 24 h after seeding, cells were treated with a single dose (1000 nM) of each compound for 3 days, after which cells were fixed and stained to identify cell nuclei. Cell nuclei counts were quantified from images and normalised against vehicle-treated wells.

Univariate analysis of normalised nuclei counts across screening plates, alongside quality control and normalised Z-score distributions, are shown in fig. S1; Z prime analysis was rated good, ranging from 0.46 to 0.92. Z-Z-score plots and hierarchical clustering, comparing hits across cell lines, highlighted VOA1056 as highly resistant to many of the compounds (fig. S1). In total, 72 agents (4.5%) were identified as hits from the screen based on nuclei count (>50% reduction in nuclei count compared to vehicle controls across ≥4 of 6 assayed cell lines; Z-score < −5.5). Of these, 27 represented cytotoxic chemotherapeutics (table S2), 4 were agents of known efficacy in LGSOC (2 MEK inhibitors, trametinib and selumetinib; 2 aromatase inhibitors, letrozole and formestane), and 4 were agents duplicated across the two component libraries, leaving 37 agents of potential interest. Of these, 16 agents were prioritised for further investigation to represent the range of drug classes and mechanisms of action (table S2).

3.2. Hit validation

Dose-response analysis was performed using an 8-point 1:3 dilution series of drug concentrations across each of the 6 cell lines included in the high throughput screen. IC50 concentrations were calculated to assess sensitivity of each cell line to the agent of interest. Validated hits were defined as drugs demonstrating IC50 < 1000 nM across ≥4 of the 6 cell lines. 5 of 16 agents failed validation (abemaciclib, CDK4/6 inhibitor; maprotiline, tetracyclic antidepressant; mizolastine, anti-histamine; neratinib, tyrosine kinase inhibitor; olmutinib, epidermal growth factor receptor inhibitor). 11 agents passed hit validation: alendazole, anti-parasitic agent; carfilzomib, proteasome inhibitor; dasatinib, tyrosine kinase inhibitor; dinaciclib, CDK1/2/5/9 inhibitor; disulfiram, aldehyde dehydrogenase (ALDH) inhibitor; homoharringtonine, protein synthesis inhibitor; ixazomib, proteasome inhibitor; panobinostat, histone deacetylase (HDAC) inhibitor; romidespin, HDAC inhibitor; selinexor, nuclear export inhibitor; volasertib, polo-like kinase inhibitor.

3.3. Comparison with HGSOC cells

To identify agents with specificity for LGSOC cells, dose-response analysis was expanded to a larger panel of 9 LGSOC cell lines and IC50 values were compared against a panel of 5 HGSOC cell lines (OVCAR3, OVS409, OVCAR4, 59M and SNU119) (Fig. 3, Fig. 4, table S3, appendix A). Analysis of trametinib sensitivity as a benchmark for an agent of known efficacy was also assessed using this approach, demonstrating a range of sensitivity across LGSOC lines, with a large proportion demonstrating greater sensitivity versus to the HGSOC comparator panel (Fig. 3E). The KRAS-mutant models HOC7 and VOA7681 demonstrated marked sensitivity to trametinib (IC50 5 nM and 24 nM) (Fig. 3E).

Of the 11 agents of interest, 7 demonstrated a low dynamic range of sensitivities with poor selectivity for LGSOC over HGSOC cells (selinexor, volasertib, panobinostat, alendazole, ixazomib, homoharringtonine, dinaciclib) (Fig. 4). Disulfiram demonstrated a range of sensitivity across cell lines with favourable selectivity for LGSOC over HGSOC models (P = 0.003) (Fig. 3B, fig. S2). Dasatinib also demonstrated a range of sensitivities, with two broad groups of models (sensitive vs more resistant) (Fig. 3A). Carfilzomib (proteasome inhibitor) and romidespin (HDAC inhibitor) demonstrated a range of sensitivities across models, with a number of LGSOC cell lines demonstrating high sensitivity to these agents (IC50 < 10 nM) (Fig. 3C and D).

Overall, VOA1056 and HO433 were highly resistant to most agents; hierarchical clustering of IC50 values across compounds grouped these cell lines alongside the HGSOC comparator cells (Fig. 4H).

3.4. Synergistic combinations with trametinib

The four agents of greatest interest (disulfiram, dasatinib, carfilzomib, romidespin), representing compounds with distinct mechanisms of action, were taken forward into combination assays with trametinib to identify potential synergistic combinations. Combination assays were performed as a 6 × 6 dose-ratio matrix across four cell lines representing a spectrum of RAS/RAF-mutation status and trametinib sensitivities (RAS/RAF-mutant: HOC7, trametinib IC50 5 nM and VOA7681, trametinib IC50 24 nM; RAS/RAF-wildtype: VOA13738, trametinib IC50 24 nM and HO433, trametinib IC50 > 1000 nM).
Dasatinib demonstrated marked synergy with trametinib in VOA7681 cells with a maximum zero interaction potency synergy score (ZIP-max) of 46.9 (Fig. 5A). Dasatinib also demonstrated notable synergy with trametinib in HOC7 cells (ZIP-max 15.4) and VOA13738 cells (ZIP-max 19.8) across multiple dose combinations; no evidence of dasatinib-trametinib synergy was identified in the RAS/RAF-wildtype, trametinib-resistant HO433 cells (ZIP-max 1.6). Disulfiram demonstrated evidence of synergy with trametinib in VOA7681, VOA13738 and HO433 cells (ZIP-max 17.7, 20.6 and 18.0, respectively), but this occurred across relatively narrow dose ranges (Fig. 5B). Disulfiram did not demonstrate evidence of synergy with trametinib in HOC7 cells (ZIP-max 7.0).

Carfilzomib did not demonstrate synergy with trametinib across multiple dose combinations in any of the tested models (Fig. S3). Romidepsin demonstrated evidence of synergy across multiple dose combinations in HO433 cells (ZIP-max 34.7), but this appeared limited to the highest trametinib doses. No evidence of romidepsin-trametinib synergy was identified in HOC7 or VOA7681 (ZIP-max 2.2 and 6.4) (Fig. S4); synergy scores in VOA13738 suggested a romidepsin-trametinib combinatorial effect at the threshold between additivity and synergy in VOA13738 (ZIP-max 11.5).

**3.5. Selective SRC inhibition is synergistic with trametinib**

As dasatinib inhibits SRC family kinase (SFK) members, ABL, c-KIT and a large number of other tyrosine kinases [27], we sought to further elucidate the mechanism-of-action of synergy between dasatinib and trametinib. Several potent inhibitors of ABL and c-KIT were included in the compound library used in our screen (nilotinib, bosutinib, imatinib, pazopanib), but none of these agents were identified as hits across any of the screened cell lines. We therefore investigated whether combining a selective SFK inhibitor produced a similar synergy profile with trametinib. A novel, highly selective SFK inhibitor – NXP900 (formerly known as eCF506) [23,24] – demonstrated markedly similar synergy profiles to dasatinib across LGSOC models (Fig. 6). High levels of synergy were identified in VOA7681 (ZIP-max 41.7), notable synergy was identified in HOC7 (ZIP-max 17.1) and VOA13738 (ZIP-max 18.3), while no synergy was apparent in the trametinib-resistant HO433 cell line (ZIP-max 2.4).

**4. Discussion**

LGSOC is an uncommon and chemoresistant ovarian cancer type that typically affects younger women [2]. While recent advances have expanded treatment options for LGSOC [15] (endocrine agents [13,28], MEK inhibitors [12,14]), intrinsic and acquired resistance highlights the need for additional treatment options. Moreover, while the MEK inhibitor trametinib represents the only drug with positive randomised late-phase trial data in this disease, treatment is associated with significant toxicity, impacting patient quality of life [12]. Identifying agents that act synergistically with trametinib may increase the magnitude and duration of response to this therapy, while also allowing dose reduction to avoid treatment-related toxicities. Notably, poly-(ADP-ribose) polymerase (PARP) inhibitors – which have represented...
Fig. 3. Sensitivity of low grade serous ovarian carcinoma (LGSOC) cell lines and comparator high grade serous ovarian carcinoma (HGSOC) lines to (A) dasatinib, (B) disulfiram, (C) carfilzomib, (D) romidepsin and (E) trametinib. $N = 9$ data points per dose per cell line ($N = 3$ biological repeats of technical triplicates). Shaded areas represent confidence bands for the fitted curves; for disaggregated data, see appendix A. For calculated IC50 values, see table S3.
Fig. 4. Sensitivity of low grade serous ovarian carcinoma (LGSOC) cell lines and comparator high grade serous ovarian carcinoma (HGSOC) lines to (A) albendazole, (B) dinaciclib, (C) homoharringtonine, (D) ixazomib, (E) panobinostat, (F) selinexor, (G) volasertib. (H) Hierarchical clustering of IC50 values across cell lines. Bar above heatmap shows cell line annotation: KRAS-mutant LGSOC cells (red), NRAS-mutant LGSOC cells (orange), RAS/RAF-wildtype LGSOC cells (yellow); high grade serous ovarian carcinoma (HGSOC) comparator cells (blue). N = 9 data points per dose per cell line (N = 3 biological repeats of technical triplicates). Shaded areas represent confidence bands for the fitted curves; for disaggregated data, see appendix A. For calculated IC50 values, see table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Fig. 5. Synergistic combination analysis of dasatinib and disulfiram with trametinib. (A) Zero interaction potency (ZIP) synergy scores for dasatinib and trametinib across low grade serous ovarian carcinoma (LGSOC) cell lines. (B) ZIP synergy scores for disulfiram and trametinib across LGSOC cell lines. N = 9 data points per dose combination (N = 3 biological repeats of technical triplicates). HOC7 and VOA7681: KRAS-mutant; VOA13738 and HO433: KRAS/NRAS/BRAF wildtype.
a step-change in the management of HGSOC [29] – are not considered to be of interest in LGSOC due to the absence of homologous recombination repair defects in this tumour type [2,30].

Our high throughput screen identified a relatively large number of compounds with substantial efficacy (>50% reduction in nuclei counts versus vehicle) across over half of the cell lines included. These hits included multiple agents of known efficacy in LGSOC, including trametinib and selumetinib. Our hits included several drugs with shared mechanisms-of-action, including multiple proteasome inhibitors, multiple MEK inhibitors, and multiple HDAC inhibitors. 16 compounds were prioritised for dose-response hit validation studies using resupplied compounds. 11 of these compounds passed validation analysis. As our screen included only LGSOC cells without comparator lines, we expanded dose-response profiling of our 11 agents of interest to a total of 9 LGSOC lines and a panel of 5 HGSOC comparator lines to identify agents that may be selective for LGSOC cells. Using the dose-response profile of trametinib as a reference for a drug of known interest and clinical activity, we identified four compounds of greatest interest based on a spectrum of sensitivity across lines with or without selectivity for LGSOC over comparator cells: dasatinib, a tyrosine kinase inhibitor; disulfiram, an ALDH inhibitor; carfilzomib, a proteasome inhibitor; and romidepsin, an HDAC inhibitor.

Disulfiram showed excellent selectivity for LGSOC, with cell lines showing uniformly higher sensitivity versus the HGSOC comparator cells. Disulfiram is currently used for management of alcohol dependency, irreversibly inhibiting ALDH to induce acute alcohol sensitivity; however, it has recently received research attention for potential utility as an anti-cancer agent [31]. While some investigators have attributed disulfiram's anti-cancer action to its ALDH-inhibitory activity [32], many others have suggested alternative anti-cancer mechanisms, such as intracellular copper accumulation and associated cell death [20,31]. Other proposed mechanisms include inhibition of angiogenesis, interference with redox homeostasis and inhibition of drug efflux pumps [31].

Synergy analysis with trametinib identified dasatinib as a potentially useful compound for combination treatment, with multiple LGSOC lines demonstrating favourable synergy scores across multiple dose combinations. Synergy was observed in both RAS/RAF-mutant and RAS/RAF-wildtype models, though the effect was not seen in HO433 cells, which are RAS/RAF-wildtype and demonstrate high intrinsic resistance to trametinib (IC50 > 1000 nM). Dasatinib inhibits a wide range of kinases, including SFKs, ABL, c-KIT and many others. To investigate whether the synergistic relationship to trametinib was related to its SFK inhibitory action, we performed trametinib combination studies.
with NXP900, a highly selective SFK inhibitor currently undergoing first-in-human phase I dose-escalation studies [23,24], NXP900’s selectivity for SFK is in part due to its unique binding mode, as it blocks SRC and its family members in their native inactive conformation [23]. The highly divergent structures of inactive kinase conformations make NXP900 only optimal for SFK binding. In contrast, dasatinib binds to its targets in their active conformation, which exhibits significant structural homology across receptor and non-receptor tyrosine kinases. Comparison of dasatinib and NXP900 synergy profiles with trametinib revealed a striking correlation, strongly suggesting SFK inhibition is a key driver of dasatinib’s synergy with trametinib.

Combining MEK inhibition with other small molecule inhibitors is already an area of keen interest in LGSOC. A phase I evaluation of combining the dual RAF/MEK inhibitor avutamibotin with defactinib, a focal adhesion kinase (FAK) inhibitor, reported an impressive response rate of 46% in 24 LGSOC patients [33], and the response rate of this combination was replicated in the phase II RAMP-201 study [34]. Combined FAK and MEK inhibition is of interest across multiple RAS/RAF-driven tumour types [35,36], and FAK/integrin signalling has been implicated in MEK inhibitor resistance [37,38]. Activated FAK is pro-tumorigenic, promoting cell survival through a variety of pathways identified across multiple cancer types, including through Hippo and WNT pathway activation, and modulation of the tumour microenvironment [38]. Early in FAK signalling activation, autophosphorylated FAK acts as a scaffold, recruiting SRC and other family members into a complex that phosphorylates additional FAK tyrosine residues to mediate downstream signalling from focal adhesions. Together with our in vitro data, the known cooperation between SRC and FAK to mediate pro-tumourigenic signalling [38] – alongside the early phase trial data demonstrating favourable responses to combined FAK and MEK inhibition [33] – suggest that combined SRC and MEK inhibition may be a feasible strategy of clinical interest in LGSOC. A key consideration for combination therapy is overlapping toxicity; hypertension, fatigue, anaemia, skin rash, nausea and diarrhoea have been identified as the most frequent grade 3 or 4 adverse events associated with trametinib treatment in LGSOC [12]. Based on adverse reaction data from chronic myeloid leukaemia [39], haematological toxicity is likely to represent the most relevant potential overlapping toxicity between trametinib and dasatinib. However, identified synergy between these compounds may enable dose reduction to avoid such toxicities. Validation of next generation SFK inhibitors with significantly reduced off-target activity (such as NXP900) may provide future opportunities for refined SRC-MEK inhibitor combination strategies with high tolerability.

A major strength of this study is that we specifically utilized a compound library enriched for FDA-approved agents to identify potential opportunities for drug repurposing. Drugs already in use for other cancer types – such as NXP900 – may provide future opportunities for refined SRC-MEK inhibitor combination strategies with high tolerability.

5. Conclusion

We identify dasatinib and disulfiram as the most promising agents for clinical investigation in LGSOC. The established dosing schedules and toxicity profiles of these agents from their existing indications presents the opportunity to fast-track these drugs into clinical studies. Dasatinib demonstrates synergy with the MEK inhibitor trametinib, and corresponding synergy in a highly selective SFK inhibitor highlights SFK inhibition as the likely driver of this synergy. Synergistic combinations with trametinib may enable dose reduction to avoid toxicities associated with MEK inhibition, while also increasing overall response rates.

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CRediT authorship contribution statement


Data availability

We are happy to provide all data presented in this manuscript upon reasonable request to the corresponding author.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: RH: consultancy fees from GlaxoSmithKline and DeciBio, RE: none. JCD: none. NL: none. RM: none. LJS: none. AJO: research funding from AstraZeneca (jointly funded Clinical Research Training Fellowship with Cancer Research UK) outside the scope of this work. HK: none. MLF:
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Appendix A. Supplementary data

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References