Supplementary information S1: Novel targets in ovarian cancer

Target  | Background | Role in oncogenesis | Preclinical/clinical rationale |
---------|------------|----------------------|-------------------------------|
Src family | Involved in various cellular signal transduction pathways, regulating processes such as cell division, motility, adhesion, angiogenesis, and survival\(^1\) | First protooncogene to be identified\(^2\) | Overexpressed and activated in a high proportion of human ovarian cancer cell lines\(^3\) |
 | Nonreceptor tyrosine kinase activated through extracellular stimulation by growth factors, hormones and integrins | Key component of signalling pathways that regulate a range of key tumour cell functions, including proliferation, disruption of cell-cell contacts, migration, invasiveness, resistance to apoptosis, and angiogenesis\(^2,4\) | Src gene activation expression signature is associated with drug resistance and poor prognosis\(^4\) |

Ras-Raf-MEK-MAPK pathway

There are three known Raf isoforms (Raf-1, A-Raf and B-Raf)\(^10\)

Important intracellular signalling pathway that regulates a range of cellular functions, including cell proliferation, cell cycle regulation, survival, angiogenesis, and cell migration

Activated by ligand binding to a receptor tyrosine kinase at the cell surface, or by other receptors such as integrins, serpentine receptors, G-proteins, and cytokine receptors

The Ras proteins were first identified as the transforming component of oncogenic viruses for K-Ras and H-Ras, whereas N-Ras was identified as the transforming component of a neuroblastoma.

Stimulate tumour growth through production of matrix degrading metalloproteinases (MMPs) and mediate drug resistance\(^10\)

Activating mutations of either B-Raf or KRAS are key in the development of low-grade serous and endometrioid ovarian tumours \(^11,12\)

The Raf isoform Raf-1 regulates cell growth in ovarian cancer cells and may be important in high grade serous ovarian cancers\(^13\)

Point mutations in codons 12, 13, and 61 of Ras genes have been reported in human ovarian cancer\(^14\)

High B-Raf expression correlates with improved survival, while high Raf-1 expression is associated with poor prognosis\(^13\)
### Aurora kinase family

Comprises Aurora-A, -B and -C serine-threonine kinases

Diverse roles in the regulation of cell cycle events, including mitotic entry, centrosome function, mitotic spindle formation, chromosome biorientation and segregation, and cytokinesis

Aurora kinase dysfunction results in an inability to maintain a stable chromosome content, which may contribute to tumourigenesis

Aurora-A is amplified in 15-25% of ovarian cancer cell lines and primary tumours

Aurora A is overexpressed in over 50% of ovarian cancers

Aurora-A overexpression is associated with centrosome amplification and poor survival.

MK-0457, a pan-Aurora kinase inhibitor significantly reduces tumour burden and cell proliferation and increases tumour cell apoptosis in preclinical models of ovarian cancer

### Histone deacetylases (HDACs)

The HDAC family comprises 18 isoenzymes, which may be classified into four classes

HDACs are enzymes responsible for deacetylating histones, leading to chromatin changes which regulate transcription and other nuclear events

Class I HDACs are highly expressed in most ovarian cancers

High level of HDAC expression is associated with poor prognosis in ovarian endometrioid cancer.

HDAC inhibitors (HDACi) result in cellular apoptosis, cell cycle arrest and differentiation

HDACi have demonstrated antitumour effects in endometrial and ovarian carcinoma cell lines

HDACi also have synergistic effects with paclitaxel- and platinum-based chemotherapeutics in vitro

A phase II trial of the HDACi belinostat in platinum resistant and micropapillary/borderline (LMP) ovarian cancer patients was well tolerated and showed promising activity, with 1 partial response
HSP90
Heat shock proteins (HSPs) are a major class of molecular chaperones that play a key role in the cellular stress response and cancer. HSP90 is a molecular chaperone with a key role in maintaining the conformational stability and function of oncogenic "client" proteins, which are involved in cellular proliferation, cell cycle regulation, apoptosis, invasion, angiogenesis, and metastasis.

HSP90 inhibition leads to the simultaneous depletion of multiple proteins involved in cancer, thus blocking malignant progression at multiple levels and reducing the likelihood of drug resistance.

Hsp90 inhibitor geldanamycin increases the sensitivity of resistant ovarian adenocarcinoma cell line to cisplatin.

The HSP90i 17AAG sensitised ovarian tumour cells with constitutively active AKT to paclitaxel chemotherapy.

17AAG and carboplatin have additive growth inhibitory effects in vitro and antitumour effects were seen with the combination in vivo.

There is currently a phase II trial evaluating 17AAG and Gemcitabine in advanced ovarian cancer.

Endothelins
The endothelins (ETs) are a family of three peptides: ET-1, 2 and 3 (REF 27).

ETs are involved in the maintenance of vasomotor tone, cellular proliferation, cellular repair and development.

ETs transmit their actions through 2 receptors ET_A and ET_B.

The ET-1/ET_A signalling cascade is implicated in carcinogenesis through the activation of PKC and the RAS pathway through EGFR phosphorylation, leading to the stimulation of cellular growth and mitogenesis.

ET-1 exerts anti-apoptotic effects through the activation of the PI3K-AKT pathway.

ET_A activates cell signalling processes involved in the regulation of cell migration, spread and invasion.

ET-1 protects ovarian cancer cells from paclitaxel-induced apoptosis through a bcl-2–dependent mechanism and AKT activation.

ET-1 levels are markedly raised in the ascites of ovarian cancer patients and together with ET_A, are overexpressed and activated in 85% of ovarian tumours, correlating with advanced stages of disease.

ZD4054 is an ET_A antagonist that inhibits ET-1–induced cell proliferation in ovarian cancer cells and has additive antitumour effects in combination with paclitaxel in vivo.

The combination of ZD4054 and gefitinib results in enhanced antitumour activity in vitro and in vivo.
**JAK/STAT pathway**

The Janus-activated kinase (JAK) family consists of tyrosine kinases which activate the signal transducer and activator of transcription (STAT) family.

Regulatory role in cell proliferation, survival and angiogenesis.

**STAT3 pathway activation**

STAT3 pathway activation is associated with high-graft tumours, drug resistance and induction of antiapoptotic proteins such as survivin and Bcl-XL.

**Protein kinase C (PKC) family**

Part of serine-threonine kinase family.

Key regulatory role in intracellular signal transduction for cell growth, proliferation, survival, metastases and angiogenesis.

**PKC isoforms**

PKC isoforms are expressed, amplified or overexpressed in ovarian cancer and have been shown to correlate with other markers of poor prognosis.

**Hedgehog signalling pathway**

Named after the family of extracellular hedgehog ligands, including three in mammals: sonic, Indian and desert hedgehog.

Activates the downstream Gli family of transcription factors.

**Hedgehog–Gli signalling**

Hedgehog–Gli signalling regulates the self-renewal and tumorigenesis of CD133 cancer stem cells.

**CD133**

CD133 has been detected on ovarian cancer cell lines, primary cancers and ascites.

**CD133 expression**

CD133 ovarian tumour cells have a higher clonogenic efficiency and proliferative potential compared to CD133 cells.

**CD133 expression in ovarian cancer**

CD133 expression in ovarian cancer is directly regulated by epigenetic modifications and support the hypothesis that CD133 cells are an ovarian cancer-initiating cell population.

**Phase II trial**

Phase II trial of a hedgehog inhibitor GDC-0449 (Genentech) is due to commence soon (NCT00739661).
23. Mackay, H. et al. A phase II trial of the histone deacetylase inhibitor belinostat (PXD101) in patients with platinum resistant epithelial ovarian tumors and micropapillary/borderline (LMP) ovarian...


**Useful reminder of the potential for a targeted combination approach.**


