New Therapeutic Approaches To Serous Ovarian Cancer

David Spriggs
Department of Medicine
Vice Chair for Experimental Therapeutics MKSCC
Themes for discussion

• P53 loss
  – Loss of G1 Checkpoint via p53 damage
  – Defective DNA Repair via BRCA like effect
• Stem Cells in Ovarian Cancer
• New Strategies in Immunotherapy
TARGETING THE GENOMIC CHARACTERISTICS OF OVARIAN CANCER

A) Loss of p53 function
Identification of potential synthetic lethal genes to p53 using a computational biology approach

Xiaosheng Wang* and Richard Simon*

Abstract

Background: Identification of genes that are synthetic lethal to p53 is an important strategy for anticancer therapy as p53 mutations have been reported to occur in more than half of all human cancer cases. Although genome-wide RNAi screening is an effective approach to finding synthetic lethal genes, it is costly and labor-intensive.

Methods: To illustrate this approach, we identified potentially druggable genes synthetically lethal for p53 using three microarray datasets for gene expression profiles of the NCI-60 cancer cell lines, one next-generation sequencing (RNA-Seq) dataset from the Cancer Genome Atlas (TCGA) project, and one gene expression data from the Cancer Cell Line Encyclopedia (CCLE) project. We selected the genes which encoded kinases and had significantly higher expression in the tumors with functional p53 mutations (somatic mutations) than in the tumors without functional p53 mutations as the candidates of druggable synthetic lethal genes for p53. We identified important regulatory networks and functional categories pertinent to these genes, and performed an extensive survey of literature to find experimental evidence that support the synthetic lethality relationships between the genes identified and p53. We also examined the drug sensitivity difference between NCI-60 cell lines with functional p53 mutations and NCI-60 cell lines without functional p53 mutations for the compounds that target the kinases encoded by the genes identified.

Results: Our results indicated that some of the candidate genes we identified had been experimentally verified to be synthetic lethal for p53 and promising targets for anticancer therapy while some other genes were putative targets for development of cancer therapeutic agents.

Conclusions: Our study indicated that pre-screening of potential synthetic lethal genes using gene expression profiles is a promising approach for improving the efficiency of synthetic lethal RNAi screening.

Keywords: Cancer, p53 mutations, Synthetic lethal genes, Gene expression profiles, Computational biology
Synthetic lethal testing

Diagram showing the comparison between normal cells and tumour cells in response to DNA damage. The diagram illustrates the pathways and outcomes for each type of cell.
What processes are targeted

![Bar chart showing important biological functions associated with candidate p53 synthetic lethal genes.](http://www.biomedcentral.com/1755-8794/6/30)
Candidate Genes

### Table 2 The candidate genes with synthetic lethality to p53 identified in at least two different datasets

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLK1</td>
<td>polo-like kinase 1</td>
<td>cell cycle regulation</td>
<td>[13-19]</td>
</tr>
<tr>
<td>CDK16</td>
<td>cyclin-dependent kinase 16</td>
<td>cell cycle regulation</td>
<td>[20]</td>
</tr>
<tr>
<td>RYK</td>
<td>receptor-like tyrosine kinase</td>
<td>cellular growth and differentiation Regulation</td>
<td>[21]</td>
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<tr>
<td>MTOR</td>
<td>mechanistic target of rapamycin (serine/threonine kinase)</td>
<td>cellular metabolism, growth, and proliferation Regulation</td>
<td>[22-24]</td>
</tr>
<tr>
<td>STK17B</td>
<td>serine/threonine kinase 17b</td>
<td>positive regulation of apoptosis</td>
<td>[25]</td>
</tr>
<tr>
<td>PLK4</td>
<td>polo-like kinase 4</td>
<td>cell cycle regulation</td>
<td>[17,26]</td>
</tr>
<tr>
<td>MAST2</td>
<td>microtubule associated serine/threonine kinase 2</td>
<td>cell cycle regulation</td>
<td>[27]</td>
</tr>
<tr>
<td>MAP3K4</td>
<td>mitogen-activated protein kinase kinase 4</td>
<td>role in signal transduction cascades</td>
<td>[28]</td>
</tr>
<tr>
<td>MARK2</td>
<td>MAP/microtubule affinity-regulating kinase 2</td>
<td>cell polarity and microtubule dynamics regulation</td>
<td>[29]</td>
</tr>
<tr>
<td>CDK1</td>
<td>cyclin-dependent kinase 1</td>
<td>cell cycle regulation</td>
<td>[13,15-17,30,31]</td>
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<tr>
<td>NEK2</td>
<td>NIMA (never in mitosis gene a)-related kinase 2</td>
<td>cell cycle regulation</td>
<td>[14-16,30]</td>
</tr>
<tr>
<td>PRKCSH</td>
<td>protein kinase C substrate 80K-H</td>
<td>roles in inflammation, cell growth, signaling and death</td>
<td>[32]</td>
</tr>
<tr>
<td>AURKA</td>
<td>aurora kinase A</td>
<td>cell cycle regulation</td>
<td>[33,34]</td>
</tr>
<tr>
<td>BUB1</td>
<td>mitotic checkpoint serine/threonine kinase</td>
<td>cell cycle regulation</td>
<td>[35]</td>
</tr>
<tr>
<td>CDC7</td>
<td>cell division cycle 7 homolog (S. cerevisiae)</td>
<td>cell cycle regulation</td>
<td>[30]</td>
</tr>
<tr>
<td>SRPK1</td>
<td>SRSF protein kinase 1</td>
<td>cellular growth and differentiation Regulation</td>
<td>[36]</td>
</tr>
<tr>
<td>TTK</td>
<td>TTK protein kinase</td>
<td>cell cycle regulation</td>
<td>[14-16,37,38]</td>
</tr>
<tr>
<td>VRK1</td>
<td>vaccinia related kinase 1</td>
<td>cell cycle regulation</td>
<td>[39,40]</td>
</tr>
</tbody>
</table>
Anti-Tumour Treatment

p53 as a target for the treatment of cancer

Michael J. Duffy, Naoise C. Synnott, Patricia M. McGowan, John Crown, Darran O'Connor, William M. Gallagher

A UCD Clinical Research Centre, St. Vincent's University Hospital, Dublin 4, Ireland
B UCD School of Medicine and Medical Science, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 4, Ireland
C Department of Medical Oncology, St. Vincent's University Hospital, Dublin 4, Ireland
D UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin 4, Ireland

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Triple negative breast cancer

ABSTRACT

TP53 (p53) is the most frequently mutated gene in cancer, being altered in approximately 50% of human malignancies. In most, if not all, cancers lacking mutation, wild-type (WT) p53 is inactivated by interaction with cellular (MDM2/MDM4) or viral proteins, leading to its degradation. Because of its near universal alteration in cancer, p53 is an attractive target for the development of new targeted therapies for this disease. However, until recently, p53 was widely regarded as “undruggable”. This situation has now changed, as several compounds have become available that can restore wild-type properties to mutant p53 (e.g., PRIMA-1 and PRIMA-1MET). Other compounds are available that prevent the binding of MDM2/MDM4 to WT p53, thereby blocking its degradation (e.g., nutlins). Anti-mutant p53 compounds are potentially most useful in cancers with a high prevalence of p53 mutations. These include difficult-to-treat tumors such as high grade serous ovarian cancer, triple-negative breast cancer and squamous lung cancer. MDM2/4 antagonists, on the other hand, are likely to be efficacious in malignancies in which MDM2 or MDM4 is overexpressed such as sarcomas, neuroblastomas and specific childhood leukemias. Presently, early clinical trials are ongoing evaluating the anti-mutant p53 agent, PRIMA-1MET, and specific MDM2–p53 nutlin antagonists.
Small Molecules to ‘fix’ p53

Table 3
Completed and ongoing clinical trials involving anti-p53 drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tumor type(s)</th>
<th>Phase</th>
<th>Code</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>APR-246 (PRIMA-1MET)</td>
<td>Prostate, hematological</td>
<td>I</td>
<td>NCT00900614</td>
<td>Aprea AB</td>
</tr>
<tr>
<td>APR-246^a (PRIMA-1MET)</td>
<td>Ovarian</td>
<td>Ib/II</td>
<td>NCT02098343</td>
<td>Aprea AB</td>
</tr>
<tr>
<td>RO5045337 (RG7112)</td>
<td>Solid, hematological</td>
<td>I</td>
<td>NCT00623870</td>
<td>Hoffmann-LaRoche</td>
</tr>
<tr>
<td>RO5045337 (RG7112)</td>
<td>Solid, sarcomas</td>
<td>I</td>
<td>NCT00559533</td>
<td>Hoffmann-LaRoche</td>
</tr>
<tr>
<td>CGM097</td>
<td>Solid</td>
<td>I</td>
<td>NCT01760525</td>
<td>Novartis</td>
</tr>
<tr>
<td>SAR405838</td>
<td>Liposarcoma</td>
<td>I</td>
<td>NCT01636479</td>
<td>Sanofi</td>
</tr>
<tr>
<td>SAR405838^b</td>
<td>Solid</td>
<td>I</td>
<td>NCT01985191</td>
<td>Sanofi</td>
</tr>
<tr>
<td>DS-3032b</td>
<td>Solid, lymphoma</td>
<td>I</td>
<td>NCT01877382</td>
<td>Daiichi Sankyo</td>
</tr>
</tbody>
</table>

^a In combination with carboplatin.
^b In combination with pimasertib.
TARGETING THE GENOMIC CHARACTERISTICS OF OVARIAN CANCER

a) Loss of p53 function
b) DNA repair deficiency, esp Homologous Recombination
Many Pathways to Protect DNA Integrity

Endogenous or environmental
- SAM
- Nitrosated amines and bile acids
- Dietary nitrosamines

Lesion
- O6-methylguanine
- 8-oxoguanine
- N7-meG
- N2-meA
- Uracil
- Hypoxanthine
- Xanthine
- SSB

10-30
10,000-100,000
6-4 photo-products
Cyclopurines
Bulky adducts
Base mismatches
DNA double-strand breaks
Stalled replication forks
ICL

Therapeutic
- TMZ
- Alkylating agents
- Nitrosoureas
- IR
- Radiomimetics
- TOP1 poisons
- Antimetabolites
- Cisplatin
- Carboplatin
- Nitrosoureas
- TMZ
- Nucleoside analogues
- IR
- Radiomimetics
- TOP1 poisons
- Anti-metabolites
- TMZ
- TopoI poisons
- Anti-metabolites
- Cisplatin
- Carboplatin
- Nitrosoureas
- MMC

Repair pathway
Direct repair
BER
SSB
NER
MMR
NHEJ
HRR
ICL repair

Figure 1 | Sources of DNA damage and their repair. Endogenous and environmental sources of DNA damage are shown in green boxes, with the lesions they cause in beige boxes (where known, the approximate number of the indicated type of lesion that occurs naturally in a cell each day is shown). Therapeutic DNA-damaging agents that cause the corresponding DNA lesion are shown in orange boxes. DNA repair pathways (blue boxes) repair DNA damage that is induced by endogenous and environmental DNA-damaging agents and thus protect the genome but they antagonize the efficacy of therapeutic DNA-damaging agents (except for mismatch repair (MMR)). BER, base excision repair; HRR, homologous recombination repair; ICL, interstrand crosslink; IR, ionizing radiation; MMC, mitomycin C; NER, nucleotide excision repair; NHEJ, non-homologous end joining; ROS, reactive oxygen species; SAM, S-adenosyl methionine; SSB, single-strand break; SSBR, SSB repair; TMZ, temozolomide; TOP1, topoisomerase I; UV, ultraviolet.
Key DNA pathways in Ovarian Ca
Double Stranded Breaks

Ataxia-telangiectasia mutated (ATM) is activated by DNA double-strand breaks (DSBs) and triggers the G1 checkpoint, by phosphorylating — and hence activating — CHK2 and p53. Ataxia-telangiectasia and Rad3-related (ATR) is primarily activated by junctions of single-stranded DNA and double-stranded DNA, which arise at stalled replication forks and resected DSBs and are nucleotide excision repair (NER) intermediates. This triggers the intra-S phase and the G2 checkpoints via phosphorylation of CHK1, which in turn phosphorylates WEE1 (which activates this kinase) and cell division cycle 25 (CDC25) phosphatases (which inhibits it) to inhibit cell cycle progression through the coordinate suppression of cyclin-dependent kinase (CDK) activity. It is important to note that there is crosstalk between the ATM-CHK2 and ATR-CHK1 pathways and that they share many substrates. ATRIP, ATR-interacting protein. Dashed arrows indicate secondary targets.
**Double Stranded Breaks and p53 loss**

Ataxia-telangiectasia mutated (ATM) is activated by DNA double-strand breaks (DSBs) and triggers the G1 checkpoint, by phosphorylating — and hence activating — CHK2 and p53.

Ataxia-telangiectasia and Rad3-related (ATR) is primarily activated by junctions of single-stranded DNA and double-stranded DNA, which arise at stalled replication forks and resected DSBs and are nucleotide excision repair (NER) intermediates. This triggers the intra-S phase and the G2 checkpoints via phosphorylation of CHK1, which in turn phosphorylates WEE1 (which activates this kinase) and cell division cycle 25 (CDC25) phosphatases (which inhibits it) to inhibit cell cycle progression through the coordinate suppression of cyclin-dependent kinase (CDK) activity.

Dashed arrows indicate secondary targets.
# Strategies for p53⁻/⁻ cells

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Target</th>
<th>Inhibitor</th>
<th>Current stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Checkpoints</td>
<td>ATM</td>
<td>KU55933, KU60019 and CP466722</td>
<td>Preclinical in vitro sensitization to IR, etoposide and camptothecin</td>
</tr>
<tr>
<td></td>
<td>ATR</td>
<td>Caffeine, shisandrin B, NU6027 and VE821</td>
<td>Preclinical in vitro chemosensitization and radiosensitization</td>
</tr>
<tr>
<td></td>
<td>WEE1</td>
<td>MK-1775</td>
<td>Preclinical in vitro and in vivo chemosensitization and radiosensitization and patient-derived sarcoma explants ex vivo and as a single agent. Evidence of activity in clinical trials.</td>
</tr>
<tr>
<td></td>
<td>CDC25</td>
<td>Several, including IRC-083864 (Debio 0931)</td>
<td>IRC-083864 has activity in pancreatic and prostate cancer xenografts and has entered clinical trial under the name of Debio 0931 (REF. 195) but no data are available.</td>
</tr>
<tr>
<td></td>
<td>CHK1 and CHK2</td>
<td>UCN-01</td>
<td>CHK1 and CHK2 (UCN-01 is a pan-kinase inhibitor): Phase I/II trials as a single agent and in combinations, trials were stopped owing to toxicities.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZD7762</td>
<td>CHK1 and CHK2: Phase I combinations with gemcitabine and with irinotecan.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF00477736</td>
<td>CHK1: Phase I combination with gemcitabine.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCH900776</td>
<td>CHK1: Phase I various drug combinations in leukaemia and lymphoma.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>XL9844</td>
<td>CHK1 and CHK2: Phase I in combination with gemcitabine.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LY2606368</td>
<td>CHK1: Phase I single agent trial.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PV1019</td>
<td>CHK2: in vitro sensitization of topoisomerase I poisons and IR.</td>
</tr>
</tbody>
</table>

APE1, AP endonuclease 1; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related; BER, base excision repair; CDC25, cell division cycle 25; CDK1, cyclin-dependent kinase 1; CLL, chronic lymphocytic leukaemia; DAC, decitabine; DDR, DNA damage response; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, DNA double-strand break; FEN1, flap endonuclease 1; HRR, homologous recombination repair; IR, ionizing radiation; MGMT, O⁶-methylguanine DNA methyltransferase; MMC, mitomycin C; MMR, mismatch repair; MMS, methyl methanesulphonate; MTD, maximum tolerated dose; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NIH, US National Institutes of Health; PARP, poly(ADP-ribose) polymerase; PARPi, PARP inhibitor; Pol, DNA polymerase; TLS, translesion synthesis; TMZ, temozolomide; XP, Xeroderma pigmentosum. *Where no reference is given information may be found on the ClinicalTrials.gov website (see Further information).
ATR Targeting

ATR (AtaxiaTelangectasia Rad3) kinase is activated by DSB and stalled replication forks

Ovarian Ca Genomic Instability is correlated to ATR / CHK1 amplification

Inhibition of WEE1 results in loss of the G2 checkpoint, failure to repair DNA and mitotic catastrophe following DNA damage

Krajewska M et al; Oncogene 2014

Tatewaki N et al; J PharmacolSci 2013
Rad51 and ATR/CHK1

- When HR is compromised by RAD51 shRNA, the cells are sensitized to inhibition of ATR or CHK1
**CHK1 Targeting**

ATR (Ataxia Telangectasia Rad3) kinase is activated by DSB and stalled replication forks

Ovarian Ca Genomic Instability is correlated to ATR / CHK1 amplification

McNeeley S et al; Pharmacology and Therapeutics, 2014
Phase I dose-escalation study of AZD7762, a checkpoint kinase inhibitor, in combination with gemcitabine in US patients with advanced solid tumors

Edward Sausville · Patricia LoRusso · Michael Carducci · Judith Carter · Mary F. Quinn · Lisa Malburg · Nilofer Azad · David Cosgrove · Richard Knight · Peter Barker · Sonya Zabludoff · Felix Agbo · Patricia Oakes · Adrian Senderowicz

Abstract
Purpose AZD7762 is a Chk1 kinase inhibitor which increases sensitivity to DNA-damaging agents, including gemcitabine. We evaluated the safety of AZD7762 monotherapy and with gemcitabine in advanced solid tumor patients.
Experimental design In this Phase I study, patients received intravenous AZD7762 on days 1 and 8 of a 14-day run-in cycle (cycle 0; AZD7762 monotherapy), followed by AZD7762 plus gemcitabine 750–1,000 mg/m² on days 1 and 8, every 21 days, in ascending AZD7762 doses (cycle 1; combination therapy).
Results Forty-two patients received AZD7762 6 mg (n = 9), 9 mg (n = 3), 14 mg (n = 6), 21 mg (n = 3), 30 mg (n = 7), 32 mg (n = 6), and 40 mg (n = 8), in combination with gemcitabine. Common adverse events (AEs) were fatigue [41% (17/42) patients], neutropenia/leukopenia [36% (15/42) patients], anemia/Hb decrease [29% (12/42) patients] and nausea, pyrexia and alanine aminotransferase/aspartate aminotransferase increase [26% (11/42) patients each]. Grade ≥3 AEs occurred in 19 and 52% of patients in cycles 0 and 1, respectively. Cardiac dose-limiting toxicities occurred in two patients (both AZD7762 monotherapy): grade 3 troponin I increase (32 mg) and grade 3 myocardial ischemia with chest pain, electrocardiogram changes, decreased left ventricular ejection fraction, and increased troponin I (40 mg). AZD7762 exposure increased linearly. Gemcitabine did not affect AZD7762 pharmacokinetics. Two non-small-cell lung cancer patients achieved partial tumor responses (AZD7762 6 mg/gemcitabine 750 mg/m² and AZD7762 9 mg cohort).
Conclusions The maximum-tolerated dose of AZD7762 in combination with gemcitabine 1,000 mg/m² was 30 mg. Although development of AZD7762 is not going forward owing to unpredictable cardiac toxicity, Chk1 remains an important therapeutic target.

Keywords AZD7762 · Chk1 · Solid tumors · Phase I · Safety · Pharmacokinetics
WEE1 Targeting

WEE1 is activated by DNA Damage via ATR and CHK1

WEE1 kinase inhibits CDK1 via phosphorylation at Tyrosine 15

Inhibition of WEE1 results in loss of the G2 checkpoint, failure to repair DNA and mitotic catastrophe following DNA damage

Ho k et al Cell Cycle 2013
Hirai H et al; Cancer Biol Ther 2010
Bridges KA et al; Clin Cancer Res 2011
wee1, a protein kinase, regulates the G2 checkpoint in response to DNA damage. Preclinical studies have elucidated the role of wee1 in DNA damage repair and the stabilization of replication forks, supporting the validity of wee1 inhibition as a viable therapeutic target in cancer. MK-1775, a selective and potent small-molecule inhibitor of wee1, is under clinical development as a potentiator of DNA damage caused by cytotoxic chemotherapies. We present a review of the role of wee1 in the cell cycle and DNA replication and summarize the clinical development to date of this novel class of anticancer agents.
TARGETING THE GENOMIC CHARACTERISTICS OF OVARIAN CANCER

a) Loss of p53 Function
b) Impairment of DNA repair
c) “Stem-ness” or tumor initiating cells
Defining Targets For Cancer Stem Cell Specific Therapy

Cancer stem cell specific therapy results in tumor regression.

Conventional cancer therapy can lead to tumor relapse.

The diagram illustrates the processes and outcomes involving cancer stem cells and conventional cancer therapy.
Major Problem

Defining Targets For Cancer Stem Cell Specific Therapy
ROR1 Is An Embryonic Protein That Is Not Expressed At Term

- Receptor tyrosine kinase-like orphan receptor 1
- Evolutionarily conserved, type-I membrane protein
- Expressed during embryogenesis and involved in organogenesis
- Not expressed on post-partum tissues except hematogones

Broome HE, Leuk Res. 2011, 35(10):1390-4
ROR1 is a Target Antigen in Ovarian Cancer

- ROR1 also is expressed by many solid tumors
- ROR1 is highest on those cancers that are high grade and/or poorly differentiated
  - triple negative breast cancer
  - pancreatic
  - ovarian cancer
ROR1 is a Target Antigen in Ovarian Cancer

- ROR1 is expressed by many solid tumors
- ROR1 is highest on those cancers that are high grade and/or poorly differentiated
  - triple negative breast cancer
  - pancreatic
  - ovarian cancer

Ovarian cancer stem cells express ROR1, which can be targeted for anti-cancer-stem-cell therapy

Suping Zhang1, Bing Cui1, Hsien Lai, Grace Liu, Emanuela M. Ghia, George F. Widhopf II, Zhuhong Zhang, Christina C. N. Wu, Liguang Chen, Rrongrong Wu, Richard Schwab, Dennis A. Carson2, and Thomas J. Kipps2

Moores Cancer Center, University of California, San Diego, La Jolla, CA 92039

Contributed by Dennis A. Carson, October 22, 2014 (sent for review September 15, 2014; reviewed by Raaphilde H. Felding)

Although initially responsive to chemotherapy, many patients with ovarian cancer subsequently develop relapsed and potentially fatal metastatic disease, which is thought to develop from cancer stem cells (CSCs) that are relatively resistant to conventional therapy. Here, we show that CSCs express a type I receptor tyrosine kinase-like orphan receptor (ROR1), which is expressed during embryogenesis and by many different cancers, but not normal postpartum tissues. Ovarian cancers with high levels of ROR1 had stem cell-like gene-expression signatures. Furthermore, patients with ovarian cancers with high levels of ROR1 had higher rates of relapse and a shorter median survival than patients with ovarian cancers that expressed low-to-negligible amounts of ROR1. We found that ROR1-positive (ROR1+) cells isolated from primary tumor-derived xenografts (PDXs) also expressed aldehyde dehydrogenase 1 (ALDH1) and had a greater capacity to form spheroids and to engraft immune-deficient mice than did ROR1-negative (ROR1Neg) ovarian cancer cells isolated from the same tumor population. Treatment with UC-961, an anti-ROR1 mAb, or shRNA silencing of ROR1 inhibited expression of the polycomb ring-finger oncogene, Bmi-1, and other genes associated with the epithelial–mesenchymal transition. Moreover, shRNA silencing of ROR1, depletion of ROR1+ cells, or treatment with UC-961 impaired the capacity of ovarian cancer cells to form spheroids or tumor xenografts. More importantly, treatment with anti-ROR1 affected the capacity of the xenograft to reseed a virgin mouse, indicating that targeting ROR1 may affect CSC self-renewal. Collectively, these studies indicate that ovarian CSCs express ROR1, which contributes to their capacity to form tumors, making ROR1 a potential target for the therapy of patients with ovarian cancer.

Significance

This study demonstrates that the oncoembryonic surface antigen, receptor tyrosine kinase-like orphan receptor 1 (ROR1), is expressed on human ovarian cancer stem cells (CSCs), on which it seems to play a functional role in promoting migration/invasion or spheroid formation in vitro and tumor engraftment in immune-deficient mice. Treatment with a humanized mAb specific for ROR1 (UC-961) could inhibit the capacity of ovarian cancer cells to migrate, form spheroids, or engraft immune-deficient mice. Moreover, such treatment inhibited the growth of tumor xenografts, which in turn had a reduced capacity to engraft immune-deficient mice and were relatively depleted of cells with features of CSC, suggesting that treatment with UC-961 could impair CSC renewal. Collectively, these studies indicate that ovarian CSCs express ROR1, which may be targeted for anti-CSC therapy.
Anti-ROR1 mAb UC-961 Inhibits Engraftment Of Human Ovarian Cancer Cells In Immune Deficient Mice

Ovarian Cancer Cells From UC-961 Treated Mice Are Depleted Of CSC

<table>
<thead>
<tr>
<th>Cell number</th>
<th>ALDH1+</th>
<th>ALDH1-</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>5/5 (100%)</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>50</td>
<td>5/7 (71%)</td>
<td>1/8 (13%)</td>
</tr>
</tbody>
</table>
TARGETING THE GENOMIC CHARACTERISTICS OF OVARIAN CANCER

a) Loss of p53 Function
b) Impairment of DNA repair
c) “Stem-ness” or tumor initiating cells
d) Escape from Immune Surveillance
Background: Immune checkpoint inhibitors are effective cancer treatments, but molecular determinants of clinical benefit are unknown. Ipilimumab and tremelimumab are antibodies against cytotoxic T-lymphocyte antigen 4 (CTLA-4). Anti-CTLA-4 treatment prolongs overall survival in patients with melanoma. CTLA-4 blockade activates T cells and enables them to destroy tumor cells.

Methods: We obtained tumor tissue from patients with melanoma who were treated with ipilimumab or tremelimumab. Whole-exome sequencing was performed on tumors and matched blood samples. Somatic mutations and candidate neoantigens generated from these mutations were characterized. Neoantigen peptides were tested for the ability to activate lymphocytes from ipilimumab-treated patients.

Results: Malignant melanoma exomes from 64 patients treated with CTLA-4 blockade were characterized with the use of massively parallel sequencing. A discovery set consisted of 11 patients who derived a long-term clinical benefit and 14 patients who derived a minimal benefit or no benefit. Mutational load was associated with the degree of clinical benefit (P = 0.01) but alone was not sufficient to predict benefit. Using genomewide somatic neoepitope analysis and patient-specific HLA typing, we identified candidate tumor neoantigens for each patient. We elucidated a neoantigen landscape that is specifically present in tumors with a strong response to CTLA-4 blockade. We validated this signature in a second set of 39 patients with melanoma who were treated with anti-CTLA-4 antibodies. Predicted neoantigens activated T cells from the patients treated with ipilimumab.

Conclusions: These findings define a genetic basis for benefit from CTLA-4 blockade in melanoma and provide a rationale for examining exomes of patients for whom anti-CTLA-4 agents are being considered.
Table 1. Clinical Characteristics of the Patients in the Discovery and Validation Sets, According to Clinical Benefit from Therapy.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Discovery Set</th>
<th>Validation Set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long-Term Benefit (N=11)</td>
<td>Minimal or No Benefit (N=14)</td>
</tr>
<tr>
<td>Age at start of treatment — yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>63</td>
<td>60</td>
</tr>
<tr>
<td>Range</td>
<td>39–70</td>
<td>48–79</td>
</tr>
<tr>
<td>Sex — no. of patients (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3 (27)</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Male</td>
<td>8 (73)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>Disease origin — no. of patients (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acral</td>
<td>0</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Uveal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>10 (91)</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Unknown primary</td>
<td>1 (9)</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Not available</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BRAF or NRAS mutation — no. of patients (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1 (9)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>Yes</td>
<td>10 (91)</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Lactate dehydrogenase level at start of therapy — no. of patients (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8 (73)</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Above normal</td>
<td>2 (18)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Not available</td>
<td>1 (9)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Duration of response to therapy — wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
<td>14</td>
</tr>
<tr>
<td>Range</td>
<td>42–361</td>
<td>11–23</td>
</tr>
<tr>
<td>Previous therapies — no.*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Range</td>
<td>0–3</td>
<td>0–2</td>
</tr>
<tr>
<td>Melanoma stage at time of diagnosis — no. of patients (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III C</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M1a</td>
<td>0</td>
<td>1 (7)</td>
</tr>
<tr>
<td>M1b</td>
<td>5 (45)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>M1c</td>
<td>6 (55)</td>
<td>12 (86)</td>
</tr>
<tr>
<td>Overall survival — yr†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Range</td>
<td>2.0–6.9</td>
<td>0.4–2.7</td>
</tr>
</tbody>
</table>

* Previous therapies included interleukin-2 and cytotoxic chemotherapy.
† Overall survival was calculated from the date of the first dose of ipilimumab to the date of death or censoring of data.
Melanoma Results
A block diagram illustrating the mechanism of action of Iplimunab, a CTLA-4 blocker. The diagram shows the interaction between the antigen-presenting cell, T-cell, and the peptide. 

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Nonmutant</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1494</td>
<td>YLLGSSAL</td>
<td>YLLESSAL</td>
</tr>
<tr>
<td>CR9306</td>
<td>VGSSADILY</td>
<td>VESSADILY</td>
</tr>
<tr>
<td>PR4092</td>
<td>YFPEESSAL</td>
<td>YSPEESSAL</td>
</tr>
</tbody>
</table>

B: Graph showing the polyfunctional T-cell response and IFN-γ-positive T-cell response over time for peptides TKSPFEQHI and TESPFEQHI. 

C: Graph showing the polyfunctional T-cell response and IFN-γ-positive T-cell response over time for peptides GLERGGFTF and GLEREGFTF.
Tumor Mutation Burden Forecasts Outcome in Ovarian Cancer with BRCA1 or BRCA2 Mutations

Nicolai Juul Birkbak¹,², Bose Kochupurakkal¹, Jose M. G. Izarzugaza², Aron C. Eklund², Yang Li¹, Joyce Liu³, Zoltan Szallas¹⁴, Ursula A. Matulonis³, Andrea L. Richardson⁵, J. Dirk Iglehart¹,⁶, Zhigang C. Wang¹,⁶*

Figure 1. Total number of exome mutations (Nmut) and clinical outcome in high-grade serous ovarian cancer. All patients received platinum and most received taxanes in combination. A) Tumors were separated into Nmut high and low groups defined by the median Nmut across the whole cohort and compared to the rate of chemotherapy resistance. The significance of the differences was determined by Fisher’s exact test. B) The number of mutations (Nmut) for each tumor was compared in chemotherapy resistant and sensitive patients and is shown by dot plots. Median and 25-75 percentiles are indicated by horizontal lines. P-value is derived from the Wilcoxon rank-sum test. C) Kaplan-Meier analysis compared the progression-free survival (PFS) and D) overall survival (OS) between patients with high and low tumor Nmut. Patients that were progression-free or still alive at the time of last follow-up were censored (•*). Numbers of patients at risk at each interval are given below the graphs. P-values are obtained by Log-rank test.
Ovarian Cancer Relevance?

- Mismatch Repair loss/deficiency?
  - Endometrial Ca???
- Prior PARP treatment?
- Extensive prior cytotoxic therapy?
Generation Of MUC16\textsuperscript{ecto}-targeted T cells For Treatment of Advanced Ovarian Cancer

1. Construct a chimeric antigen receptor (CAR)
2. Subclone CAR gene into a retroviral vector (SFG)
3. Transduce and expand patient T cells \textit{ex vivo}
4. Infuse transduced T cells to eradicate MUC-16\textsuperscript{ecto+} tumor

\textbf{New Therapy for Ovarian Cancer}
Promising Initial Studies of MUC16 CAR

New Therapy for Ovarian Cancer

Chekmasova et al, Clin Cancer Res 2010
MUC16$^{\text{ecto}}$ “Armored” CAR T Cells

2$^{\text{nd}}$ generation CAR: 4H1128z+IL12

MUC-CD

scFv (4H11)

5’ LTR $\rightarrow$ $V_H$ $V_L$ CD28 ζ chain $\rightarrow$ 5’ LTR

New Therapy for Ovarian Cancer
2015

Koneru, Oncoimmunology, In Press
MUC16\textsubscript{ecto} “Armored” CAR T Cells

2\textsuperscript{nd} generation CAR: 4H1128z+IL12

\begin{center}
\begin{tikzpicture}
\node (start) at (0,0) {5' LTR};
\node (end) at (6,0) {5' LTR};
\node (vh) at (3,0) {$V_H$};
\node (vl) at (3,-1) {$V_L$};
\node (cd28) at (3,-2) {CD28};
\node (zeta) at (3,-3) {$\zeta$ chain};

\draw[->] (start) -- (vh);
\draw[->] (vh) -- (vl);
\draw[->] (vl) -- (cd28);
\draw[->] (cd28) -- (zeta);
\draw[->] (zeta) -- (end);

\node (muc) at (3,1) {MUC-CD scFv (4H11)};
\node (il12) at (5,0) {IL-12 Gene};
\end{tikzpicture}
\end{center}
MUC16\textsuperscript{ecto} “Armored” CAR T Cells

2\textsuperscript{nd} generation CAR: 4H1128z+IL12

New Therapy for Ovarian Cancer
2015

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MUC16\textsuperscript{ecto} “Armored” CAR T Cells

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New Therapy for Ovarian Cancer
2015
Koneru, Oncoimmunology, In Press
MUC16\textsuperscript{ecto} “Armored” CAR T Cells

2\textsuperscript{nd} generation CAR: 4H1128z+IL12

“Armored” CAR: 4H1128zIL12

CAR inj 14 days post-tumor

CAR inj 28 days post-tumor

New Therapy for Ovarian Cancer

2015

Koneru , Oncoimmunology, In Press
Adding A Suicide Gene For Safety To Transition To Phase I Clinical Trial

Armored CAR with Suicide Gene: EGFR4H1128zIL12

Flow cytometric analysis of human T cells retrovirally transduced to express EGFR4H1128zIL12 showing co-expression CAR and EGFR on cell surface.

Efficacy of EGFRt Suicide Gene In Vivo

Koneru, Oncoimmunology, In Press