Developing Effective and Tolerable Combinations

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Disclosures

• I am a full time employee of AstraZeneca UK
• I hold AstraZeneca shares
• I will mention non-approved use of investigational agents, AZD1775, AZD2014
Principles for Combination Therapy

- Molecular signaling pathways driving cancer have feedback mechanisms which reactivate signaling and resistance mechanisms which emerge clinically under clonal selection pressure.

- Schedule matters – and needs to be optimised
  - Continuous inhibition not necessarily optimal.

- Lineage matters – understand how lineage affects likely resistance to target mechanism.
  - Agents which target lineage markers form ‘backbone’ for combinations – eg ER, AR, BTK.

- Aim is to kill cancer cells – not just inhibit proliferation
  - We need to understand why cells die or avoid cell death in reaction to therapies.

- Understand and anticipate likely tolerability issues and PK interaction.
Case Studies

Developing combinations in ER+ breast cancer

Developing DDR combinations
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Developing DDR combinations
Targeting the PI3K pathway

What have we learned?

1. **Broad Pan-PI3K agents have significant off-target toxicities**

2. **Pathway reactivation limits efficacy by reducing cancer cell kill**

3. **Continuous dosing regimens poorly tolerated**

4. **Interdependence of pathways require right combinations**
Feedback and cross talk mechanisms in breast cancer

Targeting three inter-dependent signalling pathways to increase the magnitude and durability of tumour response
Vistusertib (AZD2014) - inhibits mTORC1 & mTORC2 kinases

Feedback mechanisms reactivate pathways and drive resistance to rapalogues

- Dual mTORC1/2 blockade inhibits cell growth and can trigger apoptosis
- Rapalogues incompletely inhibit mTORC1 and activate AKT via feedback, giving only partial growth delay and leading to resistance

Sylvie Guichard et al AACR 2012

MCF7

- AZD2014 (nM)  Everolimus (nM)
- DMSO  1000  300  100  30  10  1 0
- p4EBP1 (T37/46)
- pS6 (S240/244)
- TORC1
- TORC2
- pAKT(S473)

HCC1428
LTED, everolimus resistant

Cell survival

Cell growth

TORC1

TORC2

mTORC2

AKT

4E-BP1

S6K

mTORC2

AKT

PI3K

PIP3

Rapalogues

FKBP12

S6K

p4EBP1 (T37/46)
pS6 (S240/244)
P

pAKT

pS6 (S235/236)

Cell survival

Cell growth

Vehicle 1% polysorbate p.o. q.d.
Everolimus 5 mg/kg p.o. q.d.
AZD2014 20 mg/kg p.o. b.i.d. 2 days on/5 off
Combination of fulvestrant and vistusertib (AZD2014) delivers improved efficacy in PDX ER+ breast cancer models.

CTC174
BrCa patient derived model (ER mutant)

HCC1500
ER+ BrCa model
Schedule matters: continuous vs intermittent dosing

Vistusertib (AZD2014) delivers efficacy when dosed using continuous or intermittent schedules

Higher intermittent doses induce more apoptosis and allow pathway to be ‘reset’

MCF7 model (ER+ BrCa)

Vistusertib causes apoptosis at 20mg/kg (intermittent schedule)
Clinical Target Inhibition - mTORC1 & mTORC2 inhibition - PBMCs 2hrs post dose: p4E-BP1 & pAKT

Percentage change from baseline

p4E-BP1$^{T37/46}$

50mg BD Continuous 125mg BD day 1&2

pAKT$^{S473}$

50mg BD Continuous 125mg BD day 1&2
Vistusertib + fulvestrant has 20-25% response rate in ER+ breast cancer

Intermittent dosing schedule demonstrates durable anti-tumour activity

Hamilton et al ASCO 2016
Tolerability improved on intermittent schedule

Intermittent schedule has...
- Lower incidence and severity of rash
- Lower incidence and severity of mucositis
- Higher incidence of N&V - but low % CTC grade 3

Discontinuation rate from vistusertib due to AE at the recommended Phase 2 doses is much lower on intermittent vs. continuous (5% vs. 31%)

Adverse Events of Interest in 50 mg BD Continuous and 125 mg Days 1&2 Fasted Intermittent Patients

Hamilton et al ASCO 2016
50mg continuous (n=13), 125mg intermittent (n=15)
MANTA – ongoing Phase II lead by Peter Schmid (NIHR)

A Phase II, randomized, open label study of fulvestrant in combination with the dual mTOR inhibitor AZD2014 or everolimus or fulvestrant alone in ER-positive metastatic breast cancer

ER+ve Metastatic Breast Cancer
Patients relapsed on adjuvant Al (or <12mo since end of treatment) or progressed on Al in metastatic setting
n=300

Stratify on:
• Resistance to 1st line therapy
• Measurable (vs. non measurable) disease

R 1.5:1.5:1:1

AZD2014 50mg BD + Fulvestrant 500mg 90 patients
AZD2014 170mg BD (2 days) + Fulvestrant 500mg 90 patients
Fulvestrant 500mg 60 patients
Everolimus 10 mg OD + Fulvestrant 500mg 60 patients

Post Progression Cross over to AZD2014 + Fulvestrant

Primary endpoint: PFS
Secondary endpoints: Overall survival (F vs FA), Objective response (RECIST1.1), Clinical Benefit (CR, PR or SD ≥24 weeks), Change in tumour size at 16 weeks, Duration of response, PRO (FAKT)
Translational endpoints: PI3K/Akt/PTEN mutations/deletions, PTEN by IHC, (epi)genetic markers from cfDNA
Combined mTORC1/2 and CDK4/6 inhibition causes profound effects on the CDK4/6 pathway

- AZD2014 causes effects on a number of CDK4/6 pathway markers
- The combination of AZD2014 and palbociclib causes more profound effects on some CDK4/6 pathway markers

15 Cosulich et al AACR 2015
Combining mTORC1/2 and CDK4/6 inhibitors enhances the modulation of E2F dependent genes

Cosulich et al AACR 2015
Triplet combination: targeting vistusertib + palbociclib + fulvestrant increases the magnitude and durability of tumour response

- An intermittent dosing schedule of AZD2014 in combination with palbociclib and fulvestrant causes significant tumour regression \textit{in vivo}
PASTOR – Triplet Combination Dose Escalation Schema

Background fulvestrant i.m. monthly

<table>
<thead>
<tr>
<th>AZD2014 BD – Intermittent – 2 Days On, 5 Days Off</th>
</tr>
</thead>
<tbody>
<tr>
<td>125mg (MTD+1)</td>
</tr>
<tr>
<td>100mg (MTD)</td>
</tr>
<tr>
<td>75mg (MTD-1)</td>
</tr>
<tr>
<td>50mg (MTD-2)</td>
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</table>

<table>
<thead>
<tr>
<th>Palbociclib QD 3 weeks on, 1 week off</th>
</tr>
</thead>
<tbody>
<tr>
<td>75mg (MTD-2)</td>
</tr>
<tr>
<td>100mg (MTD-1)</td>
</tr>
<tr>
<td>125mg (MTD)</td>
</tr>
</tbody>
</table>

Test both dose and schedule to enable tolerable triplet at doses known to have PD effects
Case Studies

Developing combinations in ER+ breast cancer

Developing DDR combinations
Targeting the DNA damage response in cancer

Three aspects of DDR that are different in cancer

- DDR pathway loss results in **greater dependency** on remaining DDR pathways
- Increased replication stress leads to **greater dependency** on ATR-CHK1-WEE1
- Increased levels of endogenous DNA damage and genomic instability results in **greater sensitivity** to exogenous DNA damage

*Figure adapted from O’Connor Mol. Cell 2015*
Cancer cells have aberrant DNA Damage Response

An Achilles’ heel where dependencies can be targeted selectively

**Normal DNA Damage Response**

- Slow down cell cycle to buy time

**Cancer Achilles’ Heel**

- Loss of key cell cycle checkpoints e.g. p53, CDKN2A
- Dependency on WEE1

- Repair DNA or tolerate damage

- Loss of repair e.g. BRCA
- Dependency on PARP
WEE1 inhibitor AZD1775 leads to increased activity of CDK1 and CDK2, aberrant DNA replication and premature mitosis.

**IC$_{50}$ 5 nM; EC$_{50}$ 80 nM vs pCDK1$^{Y15}$**

**1. Enhance DNA damage in G1 and S phase**
- a. Replication stress induced (overexpressed MYC, cyclins or KRASm)
- b. Chemotherapy induced
- c. Radiation induced
- d. Olaparib induced

**2. Prevent repair of damage in G2 phase**

**3. Increase DNA damage taken into M phase**
Wee1 inhibitor (AZD1775) plus olaparib combination activity in a SCLC chemo-sensitive CDX model

Data from Caroline Dive’s group (Manchester Institute, UK)

Known genetics include three cell cycle defects (p53, pRB, cyclin E amp)

![Graphs showing relative tumor volume over time for different treatments](image-url)
AZD1775 alone and in combination with olaparib in TNBC PDXs

HBCx9 (p53m)

- Vehicle
- OLP 100 qdx49
- OLP 50 (qdx5, 2 days off) x7
- AZD1775 120 (qdx5, 2 days off) x7
- OLP 50 (qdx5, 2 days off) x7
- AZD1775 120 (qdx5, 2 days off) x7

HBCx17 (BRCA2m, Cyclin E, CDKN2A) p53m

- Vehicle bid x28
- OLP 100 qdx28
- AZD1775 120 (qdx5, 2 days off) x7
- AZD1775 120 (qdx5, 7 days off) x2
- OLP 100 (qdx14 from D1-D14)
- OLP 50 (qdx14 from 28)

HBCx10 (BRCA2m, p53m)

- Vehicle qdx40
- OLP 100 qdx40
- AZD1775 120 (qdx5, 2 days off) x6
- OLP 50 (qdx5, 2 days off) x6

Tumour volume

Time (days)
AZD1775 activity alone and in combination with olaparib (best response waterfall plot) in TNBC PDX models

Olaparib

- CR/PR: 3/24 (13%)
- SD: 2/24 (8%)
- CB: 5/24 (21%)

AZD1775

- CR/PR: 5/23 (22%)
- SD: 5/23 (22%)
- CB: 10/23 (43%)

AZD1775 / Olaparib

- CR/PR: 13/23 (22%)
- SD: 3/23 (22%)
- CB: 16/23 (43%)

Violeta Serra
Christina Cruz
Judith Balmaña

Val D’Hebron Institute of Oncology
Selective DDR agents eg PARP inhibitors have wider therapeutic index than chemotherapy

Selective ➔ Better tolerated ➔ Longer treatment ➔ Higher efficacy

BRCA2m TNBC PDX model

Olaparib: Low impact on bone marrow cellularity and total cell count

Control

Olaparib 99 doses

Carboplatin 7 doses (99 days)

**P < 0.0001

[Graph showing tumor volume over time for different treatments]
Understanding the kinetics of DNA damage induction and repair in BM following olaparib and carboplatin dose

**In vivo**

**DNA damage response in bone marrow cores**

- IHC γH2AX within bone marrow following single in vivo dose

- Olaparib
  - No γH2AX signal after 24h
  - No effect on cell number

- Carboplatin
  - No γH2AX signal after 72h
  - Profound cells loss

**Nucleated cells count in bone marrow**

- Late erythroid precursors
- Early erythroid precursors
- Myeloid precursors
- Lymphoid precursors
- Non-nucleated cells

72h control

72h carboplatin
Sequenced dosing of olaparib and carboplatin can improve bone marrow tolerability

Exacerbated bone marrow combination effects can be prevented by introducing min. 48h break between carboplatin and olaparib dose

CD90 (Thy-1) expressed by hematopoietic stem cells, early myeloid & erythroid cells, immature B lymphocytes in the bone marrow

Lineages: CD45RC; CD3; CD11b; CD6; Granulocytes
Olaparib Wee1 Phase Ib combination

AZD1775 and olaparib schema*

<table>
<thead>
<tr>
<th>DAY</th>
<th>Lead-in</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1-2-3</td>
<td>1-2-3</td>
<td>4-5-6</td>
<td>8-9-10</td>
</tr>
<tr>
<td></td>
<td>AZD1775 bid D1-3AZD1775 bid D8-10</td>
<td>Olaparib (tablet) bid D1-14</td>
<td>No treatment</td>
<td></td>
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*Each cycle = 21 days
Restaging occurs every 2 cycles

Duration of treatment (N=13)

Response to treatment (N=11)

- Cohort 1: AZD1775 125 mg olaparib 100 mg
- Cohort 2: AZD1775 150 mg olaparib 100 mg
- Cohort 3 arm 1: AZD1775 175 mg olaparib 100 mg
- Cohort 3 arm 2: AZD1775 150 mg olaparib 200 mg

- Cohort 1 125 mg
- Cohort 2 150 mg
- Cohort 3 arm 1 175 mg
- Cohort 3 arm 2 150 mg
- Still on treatment

- Colon
- Prostate
- Breast
- Pheochromocytoma
- Colon
- Ovarian
- Ovarian
- Prostate
- Ovarian
- Breast
- Rectal

- Negative *gBRCAm*; high grade papillary serous;
- Negative *gBRCAm*; high grade undifferentiated;
- *BRCA* polymorphism R504 (1630 G>A); high grade papillary serous;
- *BRCAm*; poorly differentiated squamous cell carcinoma

Hamilton et al ASCO 2016
Conclusions

• Schedule matters – and needs to be optimised
  - Continuous inhibition not necessarily optimal

• Lineage matters – understand how lineage affects likely resistance to target mechanism.
  - Agents which target lineage markers form ‘backbone’ for combinations – eg ER, AR, BTK

• Aim is to kill cancer cells – not just inhibit proliferation
  - We need to understand why cells die or avoid cell death in reaction to therapies

• Understand and anticipate likely tolerability issues and PK interaction
  - Long term tolerability required
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