XVIII CONGRESSO NAZIONALE CIPOMO
Lasize, Maggio 2014

The Evolution of Medical Oncology: In search of the Achilles heels of cancers

Hilary Calvert
UCL Cancer Institute
University College London UK UK
Developments in Cancer Therapeutics

- DNA-reactive drugs
- Antimetabolites
- Targeted agents
  - Making targeted agents cancer selective
- Use of the synthetic lethal interaction to achieve selectivity

- Disclosures
  - I will consult for anyone who will listen
  - Inventors rewards on rucaparib
Mustard Gas – World War I
NITROGEN MUSTARD THERAPY
Use of Methyl-Bis(Beta-Chloroethyl)amine Hydrochloride and Tris(Beta-Chloroethyl)amine Hydrochloride for Hodgkin's Disease, Lymphosarcoma, Leukemia and Certain Allied and Miscellaneous Disorders

LOUIS S. GOODMAN, M.D.; MAXWELL M. WINTROBE, M.D.; WILLIAM DAMESHEK, M.D.; MORTON J. GOODMAN, M.D.; ALFRED GILMAN; MARGARET T. McLENNAN, M.D.

Discovery of Cisplatin

• Barnett Rosenberg (University of Michigan 1965). Was interested in the resemblance between the mitotic spindle of dividing cells and lines of magnetic force as visualized by iron filings around a magnet. (Fricker 1994)
• Passed electric currents through plates of Escherichia Coli and noted filamentous growth
• Filamentous growth due to the action of platinum compounds formed by reaction of the electrodes with ammonium in the culture medium
• Led to the development of cisplatin
Cisplatin and Carboplatin

- **Cisplatin**
  - Developed by National Cancer Institutes (USA), RMH and Johnson Matthey
  - One of the most widely used cancer drugs
    - Germ cell tumours – cured
    - Ovarian cancer – substantial increase in lifespan
    - Lung cancer, upper GI cancer, etc.
  - Lots of side effects
    - Kidneys, peripheral neuropathy, vomiting, bone marrow

- **Carboplatin**
  - Developed at ICR and RMH (London)
  - Less toxic – no kidney toxicity, little neuropathy, less vomiting. Slightly more platelets
  - One of many platinum analogues made by the Johnson Matthey Company
    - Problem of solubility solved by the cyclobutane ring
  - Clinical development (to Phase III) done under a Doctors and Dentists Exemption at RMH
    - Used in ovarian cancer, breast cancer, lung cancer, childhood cancers
Carboplatin - getting the right dose

- Launch at Imperial College 1986
- Initial indication ovarian cancer
- Clinical uptake initially slow – great variability in platelet toxicity

Anticancer drugs have a low therapeutic margin
Doses need to be close to toxic doses in order to get activity
Patients are very variable in their tolerance of drugs
Individualisation of dose is required
Area under the Curve (AUC) Based Dosing of Carboplatin

Carboplatin infusion

\[ \text{Dose} = \text{AUC} \times (\text{GFR} + 25) \]

Model solution in the form:
\[ C = A e^{-\alpha t} \]

Folic Acid Antagonists

Pteridine ring first described in *Pieris brassicae*, 1947

Folic acid levels reduced in leukaemia – supplementation (1947) caused an “acceleration phenomenon”

Methotrexate was synthesised by Yellapragada Subbarao at American Cyanamid

Methotrexate (amethopterin) was designed to antagonise folic acid
Folate-based Inhibitors of Thymidylate Synthase with Clinical Data


Raltitrexed (Tomudex™) ICR/Astrazeneca: Follow-up to CB 3717. Licensed for colon cancer in some countries. Less toxicity but still problems.

Response of Pleural Mesothelioma in Pemetrexed + Carboplatin Phase I

Responses are associated with symptomatic improvement – median duration about 1 year

Responses had previously been seen in a Phase I of pemetrexed + cisplatin (Hanauske, 1999)
Targeted Therapy: Cell Signalling Pathways

Hanahan and Weinberg (2000)
Development of receptor kinase inhibitors

- Large chemical libraries (millions of compounds)
- Robotic screening (tens of thousands per week)
- Structural biology – 3D imaging to refine the structure
- Lead optimisation – build in drug-like properties

Gefitinib (Iressa™) – one of the early targeted agents (1990s)
Chemotherapy +/- gefitinib in lung cancer: Survival (INTACT 1)

Population: ITT

Tick marks indicate censored observations

Proportion of patients surviving

Survival time (months)

Median survival (months)
1-year survival rate

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Survival (months)</th>
<th>1-year Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg/day</td>
<td>9.92</td>
<td>0.43</td>
</tr>
<tr>
<td>250 mg/day</td>
<td>9.86</td>
<td>0.41</td>
</tr>
<tr>
<td>Placebo</td>
<td>11.07</td>
<td>0.45</td>
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</table>

GOLrank p = 0.3875
Why do some “Targeted” drugs fail?

- The drug is targeted to a biological process that is present in many tissues of the body in addition to the cancer.
- The toxicity of the drug limits dose and therefore the exposure of the cancer to the drug.
- The toxicities are not confined to proliferating tissues.

Several hundred “targeted” drugs are currently in early clinical trials.
EGFR Mutations and Response to EGFR TK Inhibitors

• 33 patients treated with gefitinib or erlotinib displayed dramatic clinical regressions
  – 27 (82%) documented to have EGFR mutation
    • Missense in exons 18-21, overlapping deletions in exon 19 and small-frame insertions in exon 20

• No mutations documented in patients showing disease progression

• (Most of the mutations occur in non-smokers)

Data from Jänne et al, J Clin Oncol 23:3227, 2005
Human Genome Project
BRAF in Melanoma*

• BRAF Somatic missense mutations in 66% of malignant melanomas
• All mutations in the kinase domain
• V599E mutation in 80%
• Mutated BRAF has elevated kinase activity

* Davies et al, Nature 417:949, 2002 as part of the Cancer Genome Project, Wellcome Trust Sanger Institute
BRAF Inhibitor in malignant melanoma – response followed by relapse

- Most patients with V599E respond initially to BRAF inhibitors
- Relapse is frequently quite rapid due to the stimulation of alternative proliferative pathways
- Keratoacanthomas may occur
- Combination therapy may overcome resistance

*Slide courtesy of Jean-Pierre Armand, Institut Gustave Roussy, France*
Imatinib and the Bcr-Abl Fusion Protein

- Bcr-Abl fusion protein is a signalling kinase unique to myeloid leukaemia
- Responsible for proliferative signalling
- Imatinib inhibits the kinase activity

Imatinib produces long survival in Chronic Myeloid Leukaemia

• A fusion protein provides a target


Seven year follow up: 81% estimated event free survival
(British Journal of Haematology, 145: 40-41 Suppl. 1 Abstract: 102, 2009)
EML4-ALK
Another Fusion Protein as a Target

- Fusion protein of echinoderm microtubule-associated protein-like 4 (EML4) gene and anaplastic lymphoma kinase (ALK) gene
  
- Found in 6.7% of non-small cell lung cancer patients

1 Soda et al, Nature 448:561-6, 2007
2 Inamura K et al J Thorac Oncol 3:13-7, 2008
Activity of Crizotinib in Non-Small Cell Lung Cancer

Eunice L Kwak et al, NEJM 363:1693, 2010

Responses in patients with EML4-ALK fusions
All patients were non-smokers, 90% adenocarcinoma
Overall response rate 47/82 (57%)
Characteristics of Successful Drug Targets in Cancer Treatment

- Gene Amplification
  - Trastuzumab

- Activating mutations
  - EGFR, GIST
    - Erlotinib, gefitinib, second generation

- Fusion proteins
  - Bcr-Abl, EML4-ALK
    - Imatinib, crizotinib, second generation

- Synthetic Lethality
  - Poly(ADP-ribose)polymerase inhibitors

These targets are tumour-specific and are involved in malignant transformation or the maintenance of the malignant phenotype.

They are not epiphenomena.
DNA Repair — a process essential to cell survival

<table>
<thead>
<tr>
<th>How long is a piece of DNA?</th>
<th></th>
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<tbody>
<tr>
<td>DNA length per cell</td>
<td>2 meters</td>
</tr>
<tr>
<td>Cells per human</td>
<td>$2 \times 10^{13}$</td>
</tr>
<tr>
<td>DNA length per human</td>
<td>$4 \times 10^{13}$ meters</td>
</tr>
<tr>
<td>Distance from the Earth to the Sun</td>
<td>$1.49 \times 10^{11}$ meters</td>
</tr>
<tr>
<td>Number of return trips to the Sun</td>
<td>134</td>
</tr>
</tbody>
</table>

- Each cell sustains 10,000 to 30,000 episodes of DNA damage per day
- 5 Basic types of DNA damage — repair pathways
- Redundancy
  - Different pathways
  - 2 Alleles
MAJOR MECHANISMS OF DNA DAMAGE AND REPAIR

Ionising radiation
Polycyclic aromatic hydrocarbons
Replication errors
Ionising radiation
Oxygen radicals
Spontaneous reactions
Antitumour agents
Alkylating agents

Interstrand crosslink
Double-strand break
(6–4)PP
Bulky adduct
CPD
A–G mismatch
T–C mismatch
Insertion
Deletion
Uracil
Abasic site
8–Oxoguanine
Single-strand break
DNA alkylation
O\textsuperscript{6}–alkylguanine

Recombinational repair (HR, NHEJ)
DNA PKi
ATMi
Nucleotide excision repair
Mismatch repair
Base excision repair
PARPi
Direct reversal (AGT, MGMT)
O\textsuperscript{6}BG
PaTrin

Damage-induced DNA single-strand break

Mechanism of Action of PARP in Base Excision Repair

Nick protection → PARP-1 → Poly(ADP-ribose) synthesis → NAD⁺ → DNA repair → PARP-1 and chromatin dissociation
PARP Inhibitor Programme, Newcastle Anticancer Drug Development Initiative, 1990

• Rationale
  – Inhibition of PARP (PARP-1) known to potentiate in vitro
    • Monomethylating agents (temozolomide, nitrosoureas)
    • Topoisomerase 1 inhibitors (topotecan, irinotecan (SN38))
    • Radiation therapy

• Objective
  – To generate high affinity PARP inhibitors for in-vivo / clinical use

• Note
  – BRCA1 identified 1994, BRCA2 identified 1995
Constraining the carboxamide ring in a seven membered ring maintained the interactions with the active site.

Ki < 5nM purified full length rhPARP-1

Increased solubility
Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase

Helen E. Bryant¹, Niklas Schultz², Huw D. Thomas³, Kayan M. Parker¹, Dan Flower¹, Elena Lopez¹, Suzanne Kyle³, Mark Meuth¹, Nicola J. Curtin³ & Thomas Helleday¹,²

Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy

Hannah Farmer¹,², Nuala McCabe¹,², Christopher J. Lord², Andrew N. J. Tutt²,³, Damian A. Johnson², Tobias B. Richardson², Manuela Santarosa²,†, Krystyna J. Dillon⁴, Ian Hickson⁴, Charlotte Knights⁴, Niall M. B. Martin⁴, Stephen P. Jackson⁴,⁵, Graeme C. M. Smith⁴ & Alan Ashworth¹,²

Nature 2005; 434:913-917
(Newcastle / Sheffield)

Nature 2005; 434:917-921
(Institute of Cancer Research, London, Kudos Pharmaceuticals, Cambridge)
BRCA2-deficient cell lines are hypersensitive to PARP inhibitors (Newcastle / Pfizer Compounds)

In vitro

% survival

In vivo

Relative thigh circumference

Days after implant

“Therapeutic ratio” ~ 250

AG014699 (clinical development)

AG014361 (this expt)

Mutation in BRCA1 or BRCA2 Results in Extreme Sensitivity to PARP Inhibition (Kudos/AZ Compounds)

BRCA1

Active IC50 3.2 nM
KU0058684

Active IC50 3.4 nM
KU0058948

Inactive Analogue IC50 730 nM
KU0051529

Wild-type
Heterozygous
Homozygous

BRCA2

Log surviving fraction vs Concentration (M)

Adapted from: Farmer et al. Nature 434, 917-921, 2005
BRCA1 and BRCA2 Cancer Predisposition Genes

- Mutation carriers are predisposed to breast, ovarian, prostate, pancreatic and other cancers
- BRCA1 and BRCA2 are involved in homologous recombination repair – error-free repair of double strand breaks
- Carriers have one allele carrying a mutant, non-functioning gene. Damage to the functioning copy results in error-prone DNA repair and is oncogenic
Double Strand Break Repair

• Homologous recombination repair (HR)
  – Occurs in G2
  – Uses the opposite double strand as a template
  – Error free
  – Depends upon BCRA1 and BRCA2

• Non-homologous end joining (NHEJ)
  – Uses the enzyme DNA Protein Kinase (Ku proteins)
  – Error prone
BRCA Carriers and Cancer Susceptibility

Normal

Allele 1

\[
\begin{array}{c}
H \\
R \\
\end{array}
\]

Allele 2

\[
\begin{array}{c}
H \\
R \\
\end{array}
\]

BRCA Carrier

Allele 2

\[
\begin{array}{c}
H \\
R \\
\end{array}
\]

Allele 1 BRCA Mutation

\[
\begin{array}{c}
H \\
R \\
\end{array}
\]

DNA Damage

HR Repair

Error Free

NHEJ Repair

Error Prone

Unstable Genome

Further Mutations

Oncogenesis
Properties of Cancers Arising in BRCA1/2 Carriers

• The cancer has lost the ability to carry out HR (homologous recombination repair)

• Ovarian cancers are typically more sensitive to platinum treatment than sporadic (non-BRCA) cases.
Proposed Mechanism of PARP Inhibitors
Synthetic lethality or “belt and braces”

Without a PARP inhibitor repair occurs

Normal tissues in BRCA carrier patients have one functioning allele for BRCA.

BRCA related tumours have lost the second allele and cannot perform homologous recombination.

Persisting single strand break leads to double strand break during replication.

Homologous Recombination Error-free

BRCA1 or 2 defect
Olaparib – Kudos / AstraZeneca

- Orally available PARP inhibitor generated responses in hereditary cancers in Phase I*
- Phase II results in patients with BRCA1 or 2 related breast and ovarian cancer presented at ASCO 2009

Olaparib significantly increases progression-free survival in patients with platinum-sensitive ovarian cancer

## Preplanned subgroup analysis of progression-free survival

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Overall</td>
<td></td>
</tr>
<tr>
<td>BRCA mutation</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Status known</td>
<td></td>
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<tr>
<td>Status unknown</td>
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</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;50 yr</td>
<td></td>
</tr>
<tr>
<td>≥50 to &lt;65 yr</td>
<td></td>
</tr>
<tr>
<td>≥65 yr</td>
<td></td>
</tr>
<tr>
<td>Race or ethnic group</td>
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</tr>
<tr>
<td>White</td>
<td></td>
</tr>
<tr>
<td>Non-Jewish ancestry</td>
<td></td>
</tr>
<tr>
<td>Baseline response</td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>Partial</td>
<td></td>
</tr>
<tr>
<td>Penultimate platinum-based regimen</td>
<td></td>
</tr>
<tr>
<td>6–12 mo to progression</td>
<td></td>
</tr>
<tr>
<td>&gt;12 mo to progression</td>
<td></td>
</tr>
</tbody>
</table>

Size of circle is proportional to number of events; grey band represents 95% confidence intervals (CIs) in overall population

Will there be more tumour-selective targets?

- Cancer genome projects
- Tumour-type oriented research
- Childhood tumours
- Synthetic lethality screening
A few examples of predictive biomarkers in cancer treatment

- Breast Cancer
  - Oestrogen receptor
  - Progesterone receptor
  - Her2 amplification
- Lung Cancer
  - EGFR Mutation
  - ALK Translocation
  - KRAS mutation
- Colon Cancer
  - KRAS Mutation
- Melanoma
  - BRAF mutation

Many more to come
Using predictive biomarkers to select patients for clinical trials of new agents

Problem: If a particular mutation is present in say, 5% of patients and the trial requires 200 patients it will be necessary to screen 4,000 patients to complete the trial. This is prohibitively expensive and time-consuming.

Proposal:
- Whole genome sequencing
- Select different treatments / trials according to result
- Refer to a centre with an appropriate trial open
- Newly discovered target
Will there ever be a single target found in every patient?

- Almost certainly not!
  - Combination therapies
  - Multiplicative therapies
  - Sequential therapies
  - Multimodal therapies
Darwin – Tree of Life
65% mutations are heterogeneous and not present in every biopsy.
Acknowledgements

- Cancer Research UK
- Institute of Cancer Research / Royal Marsden Hospital
- Newcastle University
- UCL Cancer Institute
- Colleagues, collaborators and patients
- Johnson Matthey, Astrazeneca, Ali Lilly, Agouron
- The first-in Class PARP Inhibitor – Rucaparib - Newcastle Anticancer Drug Development Initiative, 1990
END

• X
Intracellular Signaling Networks Regulate the Operation of the Cancer Cell

Hanahan and Weinberg Cell 144:646, 2011
Progress in Cancer Survival 1975-2003

Figure LSU1: 5-year relative survival by site: 1975-2003

Percent surviving

Year of diagnosis

Source: SEER Program, National Cancer Institute. Incidence data are from the SEER 9 areas (http://seer.cancer.gov/registries/terms.html). Data are not age-adjusted.
And then everyone wanted an Alkylating agent… And tried to make them more selective.

Nitrogen mustard
Goodman and Gilman 1946
Yale University

Cyclophosphamide
Norbert Brock early 1950s
Asta Pharma

Melphalan
Bergel and Stock, 1954
Institute of Cancer Research, London

Target for tumour phosphatases

Natural amino acid – phenylalanine – taken up by tumours
How do they work?

DNA Double Helix

Replication fork

DNA Daughter Strands

Cross link stalls replication
Combination Chemotherapy Cures some Malignant Diseases


- **Drugs**
  - vincristine sulfate  
  - nitrogen mustard  
  - procarbazine hydrochloride  
  - Prednisone

- **Results**
  - 35 of 43 patients achieved complete remission
  - 77% of these still alive at 4 years
  - 47% were still in complete remission at 4 years
  - Approximately 38% of patients cured
 Improvement in the outlook for children with leukaemia 

The New England Journal of Medicine

TEMPORARY REMISSIONS IN ACUTE LEUKEMIA IN CHILDREN PRODUCED BY FOLIC ACID ANTAGONIST, 4-AMINOPTEROYL-GLUTAMIC ACID (AMINOPTERIN) 

Sidney Farber, M.D., Louis K. Diamond, M.D., Robert D. Mercer, M.D., Robert F. Sylvester, Jr., M.D., and James A. Wolff, M.D.

BOSTON

29 January 1948

3 April 1948

- Combination therapy
- Maintenance therapy
- Central Nervous system prophylaxis
- Multiple randomised trials

Current overall cure rate for acute lymphatic leukaemia in children is about 90%
Methotrexate cures choriocarcinoma
In 2010 there were 2543 cases of mesothelioma in the UK.

*The exposure index can be thought of as representing the number of asbestos fibres breathed by the male population in that year.*
Reduction of plasma homocysteine level following folic acid and B12 restoration in patients receiving pemetrexed
ALIMTA FOR PLEURAL MESOTHELIOMA:
SURVIVAL OF ALL ELIGIBLE PATIENTS

- ALIMTA + Cisplatin (n=226)
- Cisplatin (n=222)

HR: 0.77, p-value: 0.020

MST = 12.1 mos
MST = 13.3 mos
MST = 10.0 mos
MST = 9.3 mos

EGFR mutations associated with response are in the kinase domain

- Hypothesis
  - Activating mutation is responsible for tumour proliferation
  - Inhibited by gefitinib / erlotinib
- Gefitinib is approved for patients with activating mutations of EGFR

*Figure from Jänne et al, J Clin Oncol 23:3227, 2005*
Another Tumour-Specific Target – The Philadelphia Chromosome

Figure 2. Chromosome testing revealed translocation between one chromosome 9 and one chromosome 22, the so-called Philadelphia chromosome.

From: Tomohiro Myojo and Norihiko Hino, Internal Medicine 43: 126–130, 2004
THE BLOOD AND BONE MARROW IN YELLOW CROSS GAS (MUSTARD GAS) POISONING.

**Chart I.** Average leucocytic counts of fatal and non-fatal cases of yellow cross gas poisoning.
Formulae sell drugs

- Three formulae
- "Calvert" formula the most widely used

- Standard of care for ovarian cancer
- Widely used for
  - Lung Cancer
  - Her2 positive breast cancer
  - Paediatric cancers
  - seminoma

Merrrrill Egorin – Etienne Chatelut – Hilary Calvert
Overall Survival of Crizotinib-Treated Patients

Figure 2: Overall survival for ALK-positive, crizotinib-treated patients
Overall survival is shown for the subset of 82 ALK-positive patients who enrolled on the international, multicentre phase 1 clinical trial of crizotinib. Overall survival was calculated from the date of first crizotinib dose.

Rationale for new antifolates

F = folate
TS = Thymidylate synthase
DHFR = Dihydrofolate reductase

THYMIDINE SYNTHESIS

- dUMP
- CHFH₄
- CHOFH₄
- CH₂FH₄
- FH₂
- FH₄
- TS

Inhibited by 5-FU (as 5F-dUMP)

PURINE SYNTHESIS

- GARFT
- AICAR
- RNA

Making an inhibitor of TS will uniquely affect DNA synthesis and should work better

Making an inhibitor of Methotrexate will uniquely affect DNA synthesis and should work better
Development of High-Affinity PARP Inhibitors (Newcastle / Agouron)

- **3-aminobenzamide**
  
  \[ K_i = 4\mu M \]

- **PD128763**
  
  \[ K_i = 70nM \]

- **Hypothermia**

- **NU 1085**
  
  \[ K_i = 10nM \]

- **Rucaparib**
  
  \[ K_i = < 5 \text{ nM} \]

  *Phase I 2003, In clinical development by Clovis*

\[ \text{Agouron collaboration - crystal structure} \]
Crystal-based Drug Design of PARP Inhibitors

- Crystalization of NU1085 suggested further elaboration on the pendant benzene.
Phase 0 / 1 Trial of Rucaparib Day 1-5 Schedule with temozolomide

- Substantial (\(\geq 90\%\)) PARP inhibition seen in peripheral blood mononuclear cells and tumour biopsies
- No significant toxicities attributable to the PARP inhibitor as a single agent
- No dose-reduction for temozolomide
- Clinical activity observed

### PARP Inhibitors in Development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Company</th>
<th>Route</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rucaparib</td>
<td>Clovis</td>
<td>IV / Oral</td>
<td>Phase II</td>
</tr>
<tr>
<td>Olaparib</td>
<td>Astrazeneca</td>
<td>Oral</td>
<td>Phase II / Combo</td>
</tr>
<tr>
<td>Veliparib</td>
<td>Abbott</td>
<td>Oral</td>
<td>Phase I/II Combo</td>
</tr>
<tr>
<td>INO-1001</td>
<td>Inotek</td>
<td>IV</td>
<td>Phase I</td>
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<tr>
<td>E7016</td>
<td>Eisai</td>
<td>oral</td>
<td>Phase I</td>
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<td>CEP-9722</td>
<td>Cephalon</td>
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<td>Phase I</td>
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<td>Niraparib</td>
<td>Tesaro</td>
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<td>Phase I</td>
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<tr>
<td>BMN-673</td>
<td>Biomarin</td>
<td>Oral</td>
<td>Phase I</td>
</tr>
</tbody>
</table>
Sporadic Serious Toxicities of Antifolates

- Raltitrexed – reported drug-related deaths
  - 16/699 (2.2%) in three Phase III Trials†
- Pemetrexed
  - 4% in early Phase II trials without vitamin supplementation§

- Not possible to predict these toxicities on the basis of plasma or red-cell folate levels

† Zalcberg et al, JCO 14:716, 1996
† Cunningham et al, Ann Oncol 7:961, 1996
† Maughan et al, Proc ASCO18:Abs 1007, 1999
§ Niyikiza et al, Seminars in Oncology 29:6(Suppl 18):24, 2002
Interaction of folate and homocysteine metabolism

Homocysteine

- Methionine

CELLULAR METHYLATION REACTIONS

- Methionine Synthase (B₁₂ Dependent)

Interaction of folate and homocysteine metabolism

The plasma homocysteine level is a sensitive marker of functional folate or B₁₂ deficiency

Drug targets:
- Methotrexate *
- Raltitrexed †
- Pemetrexed ‡
Baseline homocysteine level predicts for pemetrexed-related haematological toxicity (n=267)

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
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<tbody>
<tr>
<td>BL HCYS</td>
<td>&lt;0.00001</td>
<td>0.0191</td>
<td>&lt;0.00001</td>
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<td>Tumor Type</td>
<td>0.0153</td>
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</table>

Niyikiza et al.. Mol Cancer Ther 2002 1: 545-552
Toxicities in patients receiving pemetrexed with and without folic acid and B₁₂ restoration

Adapted from Niyikiza et al.. Mol Cancer Ther 2002 1: 545-552
Olaparib Phase II in Breast Cancer

Best % change from baseline in target lesions by genotype

400 mg twice daily

100 mg twice daily

Olaparib Phase II in Ovarian Cancer

Best % change from baseline in target lesions

BRCA1 and BRCA2

- Involved in the repair of double strand breaks
  - Homologous recombination repair in G2
- Familial Breast and Ovarian Cancer
  - Carriers have a de-functioning mutation in one allele. Loss of function of the other allele causes cancer
  - ~80% lifetime risk of breast cancer
  - ~80% lifetime risk of ovarian cancer
  - Approximately 10% of cases related to BRCA1 or 2
- Prostate and pancreatic cancers
  - Also occur in BRCA1/2 carriers and are due to BRCA1/2
- BRCA2 and other cancers (JNCI 91:15 1310, 1999)

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>4.65</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3.51</td>
</tr>
<tr>
<td>Cholangio</td>
<td>4.97</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.59</td>
</tr>
<tr>
<td>Melanoma</td>
<td>2.58</td>
</tr>
</tbody>
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- Gene silencing by promoter methylation may occur in cases not occurring in carriers - ? frequency.
- BRCAAness – deficient in homologous recombination repair
  - Rosell 2006
    - FANCF was methylated in the serum DNA of 11 of 72 (15%) stage IV NSCLC patients (Rosell)
    - Suggested a relationship between promoter methylation of 14-3-3σ, sensitivity to cisplatin and BRCA1 function
    - 14-3-3σ methylated in the serum DNA of 215 of 580 stage IV NSCLC patients
Proposed mechanism and therapeutic potential

- Endogenously formed SSB are normally repaired by PARP-dependent BER.
- If PARP is inhibited, SSB persist.
- SSB form DSB at replication, which are repaired by HR.
- If HR is defective, the breaks are not repaired, and the cell dies.
- This is the first exploitation of synthetic lethality in cancer therapy.

**Diagram:***

- DNA SSB → DNA replication → DNA DSB → ATM/R → γH2AX → BRCA1, Rad50, MRE11, NBS1 → Rad 51, 52/4, RPA, ERCC1, XRCC3 → HR repair
- PARP → BER
- XRCC1 → Polβ, Lig III
- FA core complex
- FANC D2

**Synthetic lethality**
Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study

What has been achieved?

• Curative systemic therapy
  – Many leukaemias and lymphomas
  – Gestational tumours
  – Germ cell tumours (testis and ovary)
• Increase in cure rates through multimodal therapy
  – Breast cancer
  – Colon cancer
• Increase in duration of life
  – Ovarian cancer
  – Prostate cancer
  – (lung cancer)