Fanconi Anemia: Diagnosis and Treatment

Markus Grompe, MD

Oregon Health & Science University
What this talk will cover

- History (brief)
- Clinical presentation
- Cellular phenotype
- Genetics/Biochemical function
- Pathogenesis
- Treatment
- Search for new therapies
Fanconi anemia history

- Named after the Swiss Pediatrician Guido Fanconi (1892-1979)
- Guido Fanconi attended the University of Zürich. Before graduating in 1918 he trained in Lausanne, Munich, Zürich, and Bern.
- His main field of interest was in paediatrics, and in 1929 he became director of the Children’s Hospital and professor of paediatrics at the University of Zurich
- His name is attached to 17 conditions.
Fanconi Anemia

- Two major initial presentations
  - Birth defects
  - Anemia
- Typical birth defects
  - Diagnosis early in life, often before anemia
- Anemia
  - Patients usually have no/minor birth defects
  - Later presentation
**Percentage of birth defects**

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Percent in all FA patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial ray defect</td>
<td>49</td>
</tr>
<tr>
<td>Other skeletal</td>
<td>22</td>
</tr>
<tr>
<td>Renal and urinary tract</td>
<td>34</td>
</tr>
<tr>
<td>Male genital</td>
<td>20</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>14</td>
</tr>
<tr>
<td>Heart defect</td>
<td>13</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>11</td>
</tr>
<tr>
<td>CNS deformity</td>
<td>8</td>
</tr>
</tbody>
</table>
Image courtesy
Dr Blanche Alter
Anemia

- Pancytopenia/progressive bone marrow failure
  - Empty marrow on biopsy
- Initial presentation can be any blood lineage
- Age of onset: 2 -12 years
  - Rarely, if ever, presents in newborn period
- Red blood cell anemia
  - macrocytic
- Neutropenia
- Thrombocytopenia
Fanconi Anemia: Cellular phenotype

- Hypersensitivity to interstrand DNA cross-linking agents
  - Chromosome breakage, radial formation, apoptosis
  - Mitomycin C, diepoxybutane, cytoxan, psoralen + UVA
- Abnormal "G2/M" phase of the cell cycle
  - Spontaneously prolonged "G2/M"
  - "G2/M" accumulation after crosslinker treatment
- ? Oxygen sensitivity
- ? Sensitivity to inhibitory cytokines (γ-IFN, TNF-α)
Chromosome breakage read-out

<table>
<thead>
<tr>
<th>ML#</th>
<th>Ref Lab #</th>
<th>Clastogen</th>
<th>Conc ng/ml</th>
<th>Total # of Cells with the Following Breaks per Cell</th>
<th># Radials</th>
<th>% Radials</th>
<th>Total # of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>8823</td>
<td>GM0236 1</td>
<td>None</td>
<td>0</td>
<td>0  40  8  1  1  0  0  0  0  0</td>
<td>0</td>
<td>0%</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p.13</td>
<td>15</td>
<td>4  1  2  6  5  0  0  0  3</td>
<td>29</td>
<td>58%</td>
<td>50</td>
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<tr>
<td></td>
<td></td>
<td>MMC</td>
<td>150</td>
<td>7  1  0  5  2  1  1  0  3</td>
<td>30</td>
<td>60%</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DEB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### The Fifteen Fanconi Anemia Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>FA patients, estimated, %</th>
<th>Chromosome location</th>
<th>Protein product, kD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>60%</td>
<td>16q24.3</td>
<td>163</td>
</tr>
<tr>
<td>B</td>
<td>2%</td>
<td>Xp22.31</td>
<td>95</td>
</tr>
<tr>
<td>C</td>
<td>10%</td>
<td>9q22.3</td>
<td>63</td>
</tr>
<tr>
<td>D1/BRCA2</td>
<td>4%</td>
<td>13q12.3</td>
<td>380</td>
</tr>
<tr>
<td>D2</td>
<td>4%</td>
<td>3p25.3</td>
<td>155</td>
</tr>
<tr>
<td>E</td>
<td>10%</td>
<td>6p21-22</td>
<td>60</td>
</tr>
<tr>
<td>F</td>
<td>rare</td>
<td>11p15</td>
<td>42</td>
</tr>
<tr>
<td>G</td>
<td>10%</td>
<td>9p13</td>
<td>68</td>
</tr>
<tr>
<td>I</td>
<td>rare</td>
<td>15q26</td>
<td>150</td>
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<tr>
<td>J/BRIP1</td>
<td>rare</td>
<td>17q23.2</td>
<td>130</td>
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<tr>
<td>L</td>
<td>rare</td>
<td>2p16.1</td>
<td>52</td>
</tr>
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<td>M</td>
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<tr>
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FANCD2 Monoubiquitination is a Critical Event in the FA pathway

![Blot - anti-FANCD2](image)


![IF-anti-FANCD2](image)

- (mutant), (corrected)
The Fifteen FA proteins regulate DNA crosslink repair during S phase

DNA Damage with crosslink in S phase

I/D2 Associates with chromatin

I/D2 Dissociates From chromatin

E3 Ligase

DNA Repair

USP1/UAF1 activity is required for Fanconi Anemia pathway

Moldovan and D’Andrea, Ann Rev Genetics, 2009
Loading of the FA core complex on chromatin induces monoubiquitination of FANCD2/FANCI.

Monoubiquitinated FANCD2/FANCI recruit FAN1 nuclease to the damages sites, and colocalize with downstream FA proteins, possibly including RAD51C, and facilitate DNA repair.
Replication fork is stalled upon collision with ICL

Unhooking of ICL is potentially catalyzed by MUS81-EME1 and XPF-ERCC1, or newly identified FAN1 nuclease.

The unhooked lesion is bypassed by TLS polymerases
NER removes monoadducts and repairs the gap

DSB is repaired by HR, initiated by strand invasion

Resolution of recombination intermediates and repair is finished
The Fanconi Anemia DNA Repair Pathway

DNA Damage

E3 Ligase

I/D2 Associates with chromatin

I/D2 Dissociates From chromatin

DNA Repair

USP1/UAF1 activity is required for Fanconi Anemia pathway

Moldovan and D’Andrea, Ann Rev Genetics, 2009
BRCA2 is a Fanconi Anemia Gene (D1)

This Fanconi Anemia (D1) patient has two mutant \textit{BRCA2} alleles

\textbf{Conclusion:} The Breast Cancer Susceptibility Gene, \textit{BRCA2}, is the Fanconi D1 Gene (> 15 D1 families identified to date)
Paradox

In BRCA2 kindreds:

BRCA2 (+/-) Heterozygous Adult Carriers Develop Breast, Ovarian, Pancreatic Cancer (Not AML)

BRCA2(-/-) Children have Fanconi Anemia and Develop AML, Medulloblastoma, and Wilms Tumor
Fanconi anemia and somatic mosaicism

- Mosaicism
  - Not all the cells in the patient have the same genetic makeup.
  - In FA, ~20% of all patients are mosaic in their peripheral blood.
  - In addition to mutant cells they also have a population of healthy cells.
Natural history of FA

- Birth defects
- Pancytopenia
- Leukemia
- Solid tumors
Table 1. Summary of FA cohort reports

<table>
<thead>
<tr>
<th>Cohort</th>
<th>LIT</th>
<th>IFAR</th>
<th>NAS</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reporting period or date</td>
<td>1927-2001</td>
<td>1982-2001</td>
<td>2000</td>
<td>—</td>
</tr>
<tr>
<td>Total number of subjects</td>
<td>1301</td>
<td>754</td>
<td>145</td>
<td>—</td>
</tr>
<tr>
<td>Male-to-female ratio</td>
<td>1.23</td>
<td>1.05</td>
<td>1.10</td>
<td>ns</td>
</tr>
<tr>
<td>Age FA diagnosed, median (range)</td>
<td>7 (0-48)</td>
<td>na</td>
<td>5 (0-45)</td>
<td>&lt;.0002</td>
</tr>
<tr>
<td>Deceased %, at time of report</td>
<td>38%</td>
<td>38%</td>
<td>30%</td>
<td>ns</td>
</tr>
<tr>
<td>Projected median survival age, years</td>
<td>20</td>
<td>24</td>
<td>30</td>
<td>—</td>
</tr>
<tr>
<td>Leukemia, no MDS; number (% total cohort)</td>
<td>116 (9%)</td>
<td>47 (6%)</td>
<td>9 (6%)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Leukemia, cumulative incidence</td>
<td>na</td>
<td>45% by age 50</td>
<td>10% by age 24</td>
<td>—</td>
</tr>
<tr>
<td>MDS, number (% total cohort)</td>
<td>89 (7%)</td>
<td>53 (7%)</td>
<td>23 (16%)</td>
<td>.001</td>
</tr>
<tr>
<td>Solid tumor, number (% total cohort)</td>
<td>68 (5%)</td>
<td>67 (9%)</td>
<td>13 (9%)</td>
<td>.003</td>
</tr>
<tr>
<td>Solid tumor, cumulative incidence</td>
<td>na</td>
<td>36% by age 50</td>
<td>29% by age 48</td>
<td>—</td>
</tr>
<tr>
<td>Liver tumor, number (% total cohort)</td>
<td>37 (3%)</td>
<td>18 (2%)</td>
<td>2 (1%)</td>
<td>ns</td>
</tr>
<tr>
<td>Hematopoietic stem cell transplant (% total cohort)</td>
<td>220 (17%)</td>
<td>219 (29%)</td>
<td>44 (30%)</td>
<td>&lt; .0001</td>
</tr>
</tbody>
</table>

ns indicates not statistically significant; na, not available; and —, data in rows could not be subjected to test for significance.

*P value indicates significance of data at the extremes.
<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No. cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic tumors</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>50</td>
</tr>
<tr>
<td>MDS</td>
<td>53</td>
</tr>
<tr>
<td>ALL</td>
<td>5</td>
</tr>
<tr>
<td>CMMOL</td>
<td>1</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>Nonhematologic tumors</td>
<td></td>
</tr>
<tr>
<td>Liver tumors</td>
<td>18 (9)</td>
</tr>
<tr>
<td>Liver adenoma</td>
<td>11</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>6</td>
</tr>
<tr>
<td>Liver adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Brain tumors</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>4</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>1</td>
</tr>
<tr>
<td>Renal tumors</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Wilm tumor</td>
<td>4</td>
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<tr>
<td>Renal cell carcinoma</td>
<td>1</td>
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<td>Nephroblastoma</td>
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<tr>
<td>Squamous cell carcinoma</td>
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<tr>
<td>Head and neck SCC</td>
<td>19</td>
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<tr>
<td>Vulvar</td>
<td>8</td>
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<tr>
<td>Cervix</td>
<td>0</td>
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<tr>
<td>Cutaneous</td>
<td>3</td>
</tr>
<tr>
<td>Anus</td>
<td>2</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous tumors</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>1</td>
</tr>
<tr>
<td>Desmoid tumor</td>
<td>1</td>
</tr>
<tr>
<td>Gonadoblastoma</td>
<td>1</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1</td>
</tr>
<tr>
<td>Neurolemma</td>
<td>1</td>
</tr>
<tr>
<td>Osteogenic sarcoma</td>
<td>1</td>
</tr>
</tbody>
</table>

ALL indicates acute lymphoblastic leukemia; CMMOL, chronic myelomonocytic leukemia; SCC, squamous cell carcinoma.
Pathophysiology

- DNA repair
  - Bone marrow failure: damaged stem cells die
  - Cancer: unrepaired mutations cause cancer
  - Birth defects: damaged cell die
- Signaling defects
  - Bone marrow failure: cytokine hypersensitivity
  - Cancer: Clonal evasion from cytokine hypersensitivity
  - Birth defects: aberrant signaling during development
Non-canonical functions of FA proteins

A. STAT Activation

B. TNF resistance

C. Suppression of TNF over-production
Treatment of Fanconi Anemia

- Androgens
  - Rationale: males have higher hematocrits than females
- G-CSF
- Transfusions/supportive care
  - Red blood cells, platelets
- Bone marrow transplantation

Most slides are the courtesy of John Wagner, University of Minnesota
Pancytopenia

\{ 
  \begin{align*}
  & \text{HGB} < 8 \text{ g/dL} \\
  & \text{ANC} < 1000/\mu\text{L} \\
  & \text{PLT} < 40,000/\mu\text{L}
  \end{align*}
\}

- Matched Sibling HCT
- Androgens
- Cytokines
- Rx failure/toxicity or MDS/AML
- Alternate Donor HCT
Unrelated Donor BMT for FA
Survival 1987-1995

18% ± 9%

NMDP
Survival
Effect of Fludarabine

Fludarabine (n = 16)

No Fludarabine (n = 68)

P = 0.00

Years After Transplant

November 2001
New treatments are badly needed

- Gene Therapy
- Cell transplantation
- Small molecule intervention
  - Treatment
  - Prevention
    - Cancer prevention
    - Prevention of bone marrow failure
Is there a small molecule that can be beneficial to both hematopoiesis and cancer prevention?

- **Cancer prevention**
  - Enhance apoptosis of cells with damaged genomes
  - **Concern**: this could lead to loss of hematopoietic stem cells
- **Anemia prevention**
  - Enhance survival and growth of HSC
  - **Concern**: this could stimulate tumor growth
- **Best solution**
  - Prevent DNA damage or enhance DNA repair
Heterozygosity for *Trp53* accelerates tumors in *Fancd2* KO

![Graph showing epithelial cancer-free survival](graph.png)
ovarian AC
mammary AC
mammary AC
malignant fibrous histiocytoma
lung AC
malignant fibrous osteosarcoma
mammary AC
15 mo
10 mo
10 mo
Tempol Protects against Oxidative Damage and Delays Epithelial Tumor Onset in Fanconi Anemia Mice

Qing-Shuo Zhang,1 Laura Eaton,1 Eric R. Snyder,2 Scott Houghtaling,1 James B. Mitchell,3 Milton Finegold,1 Carter Van Waes,2 and Markus Grompe1
First small molecule to be tested in *Fancd2* mutant mice
Properties of tempol

- Superoxide dismutase (SOD) mimetic
- Hydroxyl radical scavenger
- Effective in animal models of ischemia-reperfusion injury
  - Myocardial infarction
  - Renal ischemia
- Effective in reducing tumor incidence in animal models of cancer
In vivo competitive repopulation assay:

1. Fancc<sup>−/−</sup>
   - NAC treatment
   - 3 mo.
   - Bone marrow

2. ROSA26<sup>Tg/+</sup>
   - Bone marrow

3. KSL cells (2K)
   - Genotyping PCR

4. Fancd2<sup>−/−</sup>
   - 1200 Rad
   - 6 months
   - Blood cells
   - Genotyping PCR
Conclusions

- The SOD mimetic tempol significantly delays tumors in an FA animal model.
- This effect is not specific for FA, but also has been seen in 3 other tumor-prone mouse models.
- Oxidative damage may be the main source of DNA damage in FA, but also other tumor models in mice.
- Tempol does not adversely affect the repopulating ability of FA mutant stem cells.
- Should we have a clinical trial with a) tempol, b) another SOD-mimetic?
Other compounds that have been tested

- Chloroquine
- N-Acetylcysteine
From:
chloroquine → ATM → oxidative stress
Mean tumor-free survival:
Placebo: 11.5 mon
Chloroquine: 12 mon
P=0.64
Models for hematopoietic defects in Fanconi Anemia

Is it really true that FA knockout mice are not a model for the anemia?
Reduced numbers of stem cells
Reduced colony forming ability

D

E
Changes in cell cycle status of stem cells

**Fancd2**
- G0-G1: 70%
- S-G2-M: 30%

**Wildtype**
- G0-G1: 87%
- S-G2-M: 13%

**Fancc**
- G0-G1: 83%
- S-G2-M: 17%

Hoechst 33342
Hematopoietic defects in Fancd2-/- mice

- Reduced numbers of KLS stem cells
- Reduced repopulation ability
- Reduced CFU-S
- Reduced colony forming ability (cobblestone assay)
- Decreased pool of quiescent (G0) stem cells
Resveratrol Improves Mitochondrial Function and Protects against Metabolic Disease by Activating SIRT1 and PGC-1α

Marie Lagouge,1,2 Carmen Argmann,1,3 Zachary Gerhart-Hines,2 Hamid Meziane,3 Carles Lerin,2 Frederic Dauvillier,6 Nadia Messadeq,3 Jill Milne,7 Philip Lambert,5 Peter Elliott,7 Bernard Geny,4 Markku Laakso,6 Pero Puigserver,2 and Johan Auwerx1,8,9,*,†
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3 Institut Clinique de la Souris, BP10142, 67404 Illkirch, France
4 Department of Respiratory, Cardiopulmonary and Exercise Physiology, Hôpitaux Universitaires, 67000 Strasbourg, France
5 Sinris Pharmaceutical, Cambridge, MA 02139, USA
6 Department of Medicine, University of Kuopio, 70211 Kuopio, Finland
7 IGBMC-ICS, 67404 Illkirch, France
8 These authors contributed equally to this work.
9 Contact: auwerx@igbmc.u-strasbg.fr
DOI: 10.1016/j.cell.2008.11.013

The SIRT1 Deacetylase Suppresses Intestinal Tumorigenesis and Colon Cancer Growth

Ron Firestein1,3,4,6,9, Gil Blander2,9,*, Shaday Michan1,9, Philipp Oberdoerffer1, Shuji Ogino3,4, Jennifer Campbell1, Anupama Bhimavarapu2, Sandra Luikenhuis1,9, Rafael de Cabo5, Charles Fuchs6, William C. Hahn6, Leonard P. Guarente2, David A. Sinclair1,9
Activators

- Resveratrol (46 μM)
- Quercetin
- Butein
- Pyrroloquinoxaline (233% at 10 μM)
- Oxazolopyridine (0.09 μM)
- SRT1720 (0.16 μM)
Regulators
NAD⁺/NADH/NAM
Protein level
Phosphorylation
AROS
DBC1

SIRT1

Brain/CNS
- Neurodegeneration

Heart
- Inflammation
- Cardioprotection

Liver
- Fatty acid oxidation
- Gluconeogenesis

WAT
- Lipogenesis

Skeletal muscle
- Insulin sensitivity
- Fatty acid oxidation

Pancreas
- Insulin secretion
SRT3025 ("SirT Diet") treatment increased the frequencies of KSL cells in both D2 mutant and wildtype mice.
Conclusions

- Fancd2 mutant mice have several hematopoietic defects, which can be used to test drugs for their potential to ameliorate the defect.
- The FA defects affect the stem cells themselves as well as the stroma.
- The Sirt1 mimetic resveratrol ameliorates some of these hematopoietic defects.
- Srt3025 significantly increases the number of stem cells in both wild-type and FA mutant mice.
Cancer Cells are often defective in one DNA Repair Pathway

Normal cells

Six normal DNA repair pathways

Cancer cells

The specific pathway lost may determine the best course of chemotherapy and radiation (personalized medicine)

BRCA1 mutation

Predicts PARPi sensitivity of breast cancer

Cancer Cells are often defective in one DNA Repair Pathway

Normal cells

\[ \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \]

+ + + + + + +

Six normal DNA repair pathways

Cancer cells

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Predicts cisplatin sensitivity of lung cancer

ERCC1 mutation

The specific pathway lost may determine the best course of chemotherapy and radiation (personalized medicine)

Cancer Cells are often defective in one DNA Repair Pathway

Normal cells

Cancer cells

Predicts TMZ sensitivity of brain cancer

Six normal DNA repair pathways

The specific pathway lost may determine the best course of chemotherapy and radiation (personalized medicine)

Using Synthetic Lethality to treat tumors with Underlying defects in the Fanconi Anemia Pathway
Since 5-20% of solid tumors in the general population have a defect in the FA pathway, we would like to find targeted therapies for these tumors.

Used the principle of “Synthetic Lethality”
Screening Approach to Identify Gene Targets in FA Cells.

**Day 1**
Cells seeded

**Day 2**
Each well transfected with siRNA oligonucleotide

**Day 6**
Measure Cell viability with ATP-activated luminiscence (quantitative measure)
**siRNA Targets Specifically Toxic to FA Pathway Deficient Cells**

<table>
<thead>
<tr>
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<tbody>
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</tr>
<tr>
<td>LIG3</td>
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</tr>
<tr>
<td>NBS1</td>
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FA Cell Lines Are Hypersensitive to the ATM Inhibitor, Ku55933

**FA-E Cells**

- **FANCE Mut**
- **FANCE Corr**

**FA-G Cells**

- **FANCG Mut**
- **FANCG Corr**

**FA-D2 Cells**

- **FANCD2 Mut**
- **FANCD2 Corr**
FA Cell Lines Are Hypersensitive to the ATM Inhibitor, Ku55933

**FA-E Cells**

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**FA-D2 Cells**

- FANCD2 Mut
- FANCD2 Corr

Synthetic Lethal Relationships of DNA repair pathways are reciprocal
Conclusions:

1) Conventional cancer treatments (Radiation and Chemotherapy) kill tumors by causing DNA damage.

2) There are six major DNA Repair pathways, and each pathway has suitable biomarkers and druggable targets.

3) There is an emerging class of DNA repair inhibitors which may be useful in cancer chemotherapy as sensitizers of conventional treatments.

4) Based on the principle of Synthetic Lethality, DNA repair inhibitors, such as Parp or Atm inhibitors, may be useful as Monotherapy for some cancers with underlying DNA repair defects.

5) Biomarkers of the FA pathway provide a convenient predictor of subsets of human tumors which are sensitive to Parp and Atm inhibitors.