Drugs for Fanconi Anemia

Richard Gelinas

Family Meeting at Camp Sunshine
Portland ME
29 June 2015
Finally: drugs against FA risks

- Drugs for healthy bone marrow (and preventing leukemia)
- Head and neck cancer: kill the cancer with minimal side-effects
- Novel approaches
- NAC: clinical trial for FA this year
- “I’m a FA parent. What can I do?”
**Glossary of small molecule terms**

**Hit:** a particular compound that has significant activity in an assay

**Lead:** a compound that is active in one or more positive assays yet is acceptably low in negative activities

**Drug candidate:** a lead compound, often with small chemical changes that improve its activity or modify its bioavailability suitable for toxicity tests in animals or phase 1 trials in people

**Bio-available:** a drug that achieves a useful concentration in the same compartment (cell, organ) as its target

**Therapeutic index:** the ratio of good things to bad things caused by a drug

**Mechanism-based toxicity:** undesirable effects of a drug acting on its target; leads to dose-limiting side effects

**N-dimensional shape space:** the diversity of small molecules (medicinal chemists say this to each other)
Old and new ways to kill tumors

**Radiation:** irradiate leading to DNA damage and cell death…a problem for FA

**Surgery:** just cut it out.

**Targeted therapy:** exploit pathways or features specific to particular tumors.

**Chemotherapy:** uses chemicals that poison all rapidly dividing cells including tumors.

**Immunotherapy:** antibodies reverse tumor camouflage.
NAC trial starting…

- Multi-center trial….to enroll 40 FA patients…starting late 2014
- FA endpoints: improve blood cell counts & reduce DNA damage
- NAC has been tried on patients with cystic fibrosis, autism and other conditions. Approved for use in children.
- NAC: inhibits oxidative stress & inflammation, by raising levels of glutathione
- NAC also in use as mucolytic agent; to treat acetaminophen overdose
- Oral use side-effects include nausea, vomiting, rash, and fever
- **Search: acetylcysteine** or NAC

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The Hospital for Sick Children and University of Toronto
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Website: [www.sickkids.ca/AboutSickKids/Directory/People/D/Yigal-Dror.html](http://www.sickkids.ca/AboutSickKids/Directory/People/D/Yigal-Dror.html)
NAC trial starting…

Study coordinator: Dr. Yigal Dror

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<tr>
<th>Institution</th>
<th>City, State/Country</th>
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<tr>
<td>1. The Hospital for Sick Children</td>
<td>Toronto, CA</td>
<td>Dr. Yigal Dror</td>
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<td>2. CHU Ste-Justine</td>
<td>Montréal, CA</td>
<td>Dr. Yves Pastore</td>
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<td>3. British Columbia Children's Hospital</td>
<td>Vancouver, CA</td>
<td>Dr. John Wu</td>
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<td>4. Giannina Gaslini Children's Hospital</td>
<td>Genova, Italy</td>
<td>Dr. Carlo Dufour</td>
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<td>5. Indiana University</td>
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NAC:

WHAT YOU NEED TO KNOW: N-ACETYL CYSTEINE’S BROAD-SPECTRUM BENEFITS

- Long relegated to infrequent use in unusual circumstances, the amino acid-derived compound N-acetyl cysteine (NAC) has drawn increased scientific attention.

- NAC replenishes levels of the intracellular antioxidant glutathione (GSH), which is often deficient with advancing age and in chronic illness.

- NAC also regulates expression of scores of genes in the pathways that link oxidative stress to inflammation.

- These dual effects give NAC a unique role in the prevention and treatment of many common diseases, both acute and chronic.

- NAC can protect against avian influenza and more common seasonal flu symptoms.

- NAC reduces the frequency and duration of attacks of chronic obstructive pulmonary disease (COPD) and may slow the clinical course of idiopathic pulmonary fibrosis (IPF).

- NAC protects tissues from the effects of exercise-induced oxidative stress, adding value and safety to your workout.

- NAC improves insulin sensitivity in people with some of the most difficult-to-treat metabolic disorders.

- NAC blocks cancer development at virtually every step in the process, and through multiple mechanisms, making it an important cancer chemopreventive agent.

- NAC fights the stomach infection Helicobacter pylori on two fronts, inhibiting the organism’s growth while reducing production of inflammatory cytokines that can lead to gastritis and cancer.

- Though most individuals gain benefits from 600-1,800 mg/day, clinical studies have found that doses of up to 2,000 mg/day are safe and effective. A recent study demonstrated the safety of 2,800 mg/day for 3 months in patients with COPD.23

Five labs pursuing FA drugs…

- **Michael Garbati** (OHSU) & Grover Bagby: drugs to prevent marrow failure (ALDH 1)
- *Markus Grompe* (OHSU): prevent marrow failure (aldehyde scavengers)
- #Ray Monnat (U.Washington): prevent marrow failure (aldehyde scavengers)
- Jordi Surrales (U. Autonoma de Barcelona): screening approved drugs in assays of cell cycle & cytogenetics; early hits reassuring: resveratrol, n-acetyl cysteine, quercertin, danazol, doramapimod
- Susanne Wells (Cincinnati Children’s Hospital): trying PARP inhibitors as anti-HNSCC therapy

#CDMRP/Army; *NIH support; **FARF support
The aldehyde hypothesis:

1. Aldehydes are naturally produced inside the cell.
2. If not chemically recycled, they damage DNA.
3. This damage is repaired very slowly in patients with FA.
4. Stem cells may die if there is too much unrepaired damage.
Before and after drinking alcohol: ‘Asian flushing syndrome’….slow recycling
Aldehyde metabolism 101

Ethanol: $R-OH \quad \text{CH}_3\text{CH}_2\text{OH}$

Acetaldehyde: $R-C=O \quad \text{CH}_3\text{CHO}$

Acetic acid: $R-C=O \quad \text{CH}_3\text{COOH}$

Enzymes involved:
- ADH (alcohol dehydrogenase)
- ALDH2 (aldehyde dehydrogenase)
- ALDH3 (aldehyde dehydrogenase)
Garbati: “… turned our focus to overexpressing ALDHs in FANCC-deficient cells to see if this also partially corrects the TLR to TNF phenotype.

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<th>Stressor</th>
<th>Stem cell&lt;sup&gt;wt&lt;/sup&gt;</th>
<th>Stem cell&lt;sup&gt;FA&lt;/sup&gt;</th>
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<tr>
<td>1. Aldehydes</td>
<td>OK</td>
<td>can’t cope</td>
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<tr>
<td>2. Cytokines</td>
<td>OK</td>
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macrophage<sup>WT</sup> | Stress, infection | Normal cytokine response |
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<th>Drug</th>
<th>Target</th>
<th>Role in FA</th>
<th>Status</th>
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<td>Alda-1 (a potential human therapeutic)</td>
<td>ALDH agonist</td>
<td>Prevent stem cell death; at least use during episodes of inflammation</td>
<td>Commercial interest; pre-clinical? Clinical trials?</td>
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Courtesy Drs. Garbati & Bagby
Aldeia Pharmaceuticals is developing a drug that speeds enzymatic removal of aldehydes for FA

ALDEA Pharmaceuticals is developing a number of small-molecule modulators of the aldehyde dehydrogenase enzyme (ALDH) superfamily.

This proprietary scientific approach was originally discovered by Stanford University researchers and exclusively licensed to Aldea. With its ALDH platform, ALDEA has the unique opportunity to pursue novel targets across multiple therapeutics areas to address significant unmet medical needs.

- Acute alcohol toxicity
- Fanconi anemia
- Alcohol metabolism deficiency (ALMD) and its long term consequences
- Other diseases and conditions associated with toxic aldehydes

Management Team

William E. Yelle, Chief Executive Officer

Bill Yelle has 25 years of experience in the life sciences industry, including positions in corporate and business development, strategic planning, portfolio management, commercial operations and R&D.
Aldehyde scavenger drugs pursued by M. Grompe and R. Monnat

Aldehyde-damaged DNA leads to bone marrow failure in FA…so test drugs that chemically neutralize aldehydes or aldehyde scavengers…in cells & mice. If these drugs are effective in FA, this work will lead to a new clinical trial.

- Ray Monnat (U. Washington): can aldehyde scavengers protect HSCs from damage or killing by aldehydes *in vitro* and *in vivo*; one is an approved drug; some clinical experience with the other lead compound.

- Markus Grompe (OHSU): testing an approved drug with *in vivo* mouse^{FA} survival assays.
FA patients at risk for head & neck cancer

- HNSCC...6th most common cancer worldwide...
  >500,000 new cases each year....survival of only 50%.
- The incidence is about 700 fold in FA patients.
- Patients with FA develop aggressive head and neck and anogenital tumors at early ages and usually succumb to the disease due to the limited treatment options.
- What causes this cancer? Is HNSCC\textsuperscript{FA} the same as typical HNSCC?
Current FARF support to: Dr. Agata Smogorzewska, Rockefeller University
$148,000 award for two years.

- “Genomic and functional analysis of the anogenital and head and neck squamous cell carcinomas (SCCs) in patients with Fanconi anemia”
- Study the genetic alterations that drive tumorigenesis in FA patients; compare to (non-FA) SCCs; define mechanism & drug targets.
- An integrated genomic, and epigenomic study of FA SCC material from International Fanconi Anemia Registry
- Submit mutations to functional assays; strong collaborations
Agata’s workplan

Samples

Sample preparation

Sequencing

Analysis

Validation

- head and neck, genital, anal squamous cell carcinomas from Fanconi anemia patients and non-tumor isogenic controls
- macrodissection, DNA, RNA extraction quantification and quality control
- whole-genome sequencing
- whole-exome sequencing
- whole-genome bisulfite sequencing
- RNA sequencing (when frozen tissue available)
- computational pipeline
- quality control
- identification of somatic point, indel, structural variants
- assessment of differential expression, gene fusions
- assessment of methylation patterns
- comparison with TCGA data
- Sanger sequencing
- FISH
- targeted sequencing of additional samples
3D models of FA SCC will be used to test drugs…in collaboration with Susanne Wells

A. Primary cells from a sporadic SCC
B. Organotypic raft derived from the primary cells shown in A.
C. Example of a tumor growing as an orthotopic xenograft in which the primary cells are implanted into the tongue of an immunocompromised mouse. In this case, the implanted SCC cells were cultured from a Fanconi anemia individual.
Genetic basis of head and neck cancer described

The Cancer Genome Atlas (http://cancergenome.nih.gov)

- Why do they develop? Which genes have mutations? What is the role of papilloma virus? Why are they so hard to cure?
- Squamous cell carcinomas (SCC) from 528 patients were analyzed by whole-exome sequencing, microarray analysis, and gene copy-number analysis.
- HPV+ HNSCC had recurrent deletions and truncating mutations in TRAF3. This gene is crucial for defense against several viruses, and its absence might impair the immune system’s ability to clear HPV.
- Some HPV-negative HNSCC had defects in a three-gene signature:
  - TP53...control of cell division and DNA repair lost
  - CASP8...control of programmed cell death lost
  - HRAS....cell division unregulated
- 1/3 of HNSCC have too many copies of the cell cycle regulator CCND1. OK there is a drug that targets this pathway. [A CDK4/6 inhibitor, Palbociclib (Ibrance; Pfizer) targets this pathway]
- Knowing the gene defects in a specific SCC should lead to choice of custom therapy. ‘precision medicine’
NCI to enroll patients in July for ‘Molecular Analysis for Therapy Choice (MATCH)’ precision medicine trial.

- Will a drug that is active against cancers with a specific mutation be effective in different types of cancer?
- 20 mutation-specific drugs (or combinations) will be matched to each patient’s tumor profile.
- 143 notorious genes will be profiled in each tumor.
“NCI-MATCH is a unique, ground-breaking trial,” said NCI’s acting director Doug Lowy, M.D. "It is the first study in oncology that incorporates all of the tenets of precision medicine. There are no other cancer clinical trials of this size and scope that truly bring the promise of targeted treatment to patients whose cancers have specific genetic abnormalities. It holds the potential to transform cancer care.”
Unleash the immune system to kill tumors...

Old ways: radiation, surgery, targeted drugs, chemotherapy
New way: unleash the immune system

‘And then there were five’. The Economist. 6 June, 2015.
The problem: tumors are hard to kill:

1. They look normal to the immune system
2. They can interfere with T cell activation
3. They can suppress the entire immune system

New drugs exploit #2 via checkpoint proteins PD-1, CTLA4

2011/ Bristol-Myers Squibb/ Yervoy/ CTLA4
2014/ Merck/ Keytruda/ PD-1
2014/ Bristol-Myers Squibb/ Opdivo/ PD-1
2015/ Roche; Astra-Zeneca following

Melanoma/ some forms of metastatic head & neck cancer, some types of lung, liver, bladder, kidney cancers
Treatment comparisons & combinations in progress.
Immunotherapy: unleash natural defense against solid tumors
Programmed cell death (PD-1) & CTLA-4 now excellent drug targets

Tumors: melanoma*, cancers of lung*, kidney*, bladder, esophagus, pancreas, head & neck
Companies: Bristol-Myers Squibb; AstraZeneca, Pfizer, Merck, others
Search: PD-1 immunotherapy; CTLA-4
*Topalian et al 2013. NEJM 366: 2443-2454
How good are these drugs?

- In untreated melanoma:
  - Combination immunotherapy with Opdivo (nivolumab) and Yervoy (ipilimumab) reduced the risk of death or disease progression by more than half compared with ipilimumab alone in a large (n=1000) randomized trial.
  - Progression, tumor growth slowed, side effects manageable.
Immune therapy for squamous cell cancer: FDA approved...will this help HNSCC in FA?

- Inhibits PD-1 & disables the brake on T cells
- “…frees the bodies own immune system to attack tumors…”
- FDA approved *Opdivo* (Bristol-Myers Squibb) for squamous cell carcinoma (SCC) of the lung.
- SCC is 1/3 of NSCLC
- Trials underway for other forms of lung cancer
- Previously approved to treat melanoma
- Huge market now: 224,000 new Dx of lung cancer 2014; 160,000 deaths; 16x worse than Ebola
- Cost: $12,500 a month
- Merck: ‘*Keytruda*’ similar, approval expected 2015
- NYTimes 5 March 2015
Presage Biosciences can test 8 drugs for tumor-killing activity.
- Promptly see what each drug does.
- Results predictive of systemic delivery.
- Entering clinical trials.

• The Presage system. (A) The syringe with eight needles.
• (B) Tumor grafted onto the flank of a mouse & injected with dye.
• (C & D) tracker dye visualized.
• (E) Remove tumor, cut sections, judge results.
• (F, G, H) Individual tumor cells resolved & show uptake of biomarker.
Dog cancerous lymph nodes targeted

- Canine tumor injected with 8 needle array (drug=vincristine)
- Tracker dye seen beneath the skin with a blue flashlight and yellow filter glasses
- Tumors were resected 24 hours after microinjection.
  - Tracker dye (green)
  - pHH3 (yellow) mitotic arrest
  - CC3 (red) cell death-apoptosis
- A low toxicity way to test new drugs in vivo, in localized way.
Circulating tumor cell (CTC) detection chip

- Cancer cells metastasize through the bloodstream either as single or groups of cells.
- The Cluster-Chip captures CTC clusters independently of tumor-specific markers from unprocessed blood.
- CTC clusters found in 30-40% of patients with metastatic breast or prostate cancer or with melanoma.
- RNA sequencing of CTC clusters confirmed their tumor origin and identified tissue-derived macrophages within the clusters.
- Efficient capture of CTC clusters will enable the detailed characterization of their biological properties and role in metastasis.
- HNSCC: early Dx? Response to Rx?

Scanning electron micrograph of a circulating tumor cell cluster captured by Cluster-Chip. [Sarioglu et al., Nature Methods] A microfluidic device for label-free, physical capture of circulating tumor cell clusters. PMID: 25984697
Healed by His Own Data

New York Times, 1 April 2015
To help yourself, get access to your own data.

- Steven Keating: brain scan - small abnormality 8 years ago…’don’t worry’
- Read all he could on brain structure & diseases
- MIT Media lab grad student
- ?Whiffs of vinegar? he identified as smell seizures…he knew his ab was near olfactory center; retest; right to surgery
- Tennis-ball sized tumor removed.
- Better-informed patients take better care of themselves.

“Data can heal. There is a huge healing power to patients understanding and seeing the effects of treatments and medications.” - S. Keating
Thanks to friends at FARF:

Laura Hays
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David Fiaschetti
Peter Pless