UPDATE ON THE FANCONI ANEMIA - N-ACETYLCYSTEINE (NAC) CLINICAL TRIAL

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Overview

- **Title**

  A prospective phase I/II study to evaluate N-acetylcysteine (NAC) for the amelioration of DNA damage, oxidative stress, and hematological anomalies in patients with Fanconi Anemia

- **Preparatory phase**

Partnering (drug company, research team)	Jan 13 - Sep 13
Protocol writing & review (2 rounds)	May 13 - May 14
Protocol and grant submission	Jun 14 - Nov 14

- **Implementation phase**

First site activated	Jan 15
End of trial	Dec 16 / Dec 17

Research Team

Sick Kids - Toronto, CANADA	**Clinical site** **Laboratory site**	 **Yigal Dror** **PI**  **David Chitayat** **Co-PI**  **Sharon Moalem** **Fellow**  **Lilian Sung** **Statistician**
Indiana Univ. - Indianapolis, USA	**Clinical site**	 **Helmut Hanenberg** **Site PI**
Univ. Minnesota - Minneapolis, USA	**Clinical site** **Laboratory site**	 **Jakub Tolar** **Site PI**
Gaslini Institute - Genoa, ITALY	**Clinical site** **Laboratory site**	 **Carlo Dufour** **Site PI**  **Johanna Svahn** **Clinician**
Emory Univ. - Atlanta, USA	**Laboratory site**	 **Rabin Tirouvanziam** **Site PI**

Rationale & hypothesis

- **Rationale**

  High oxidative stress has been documented in FA cells.

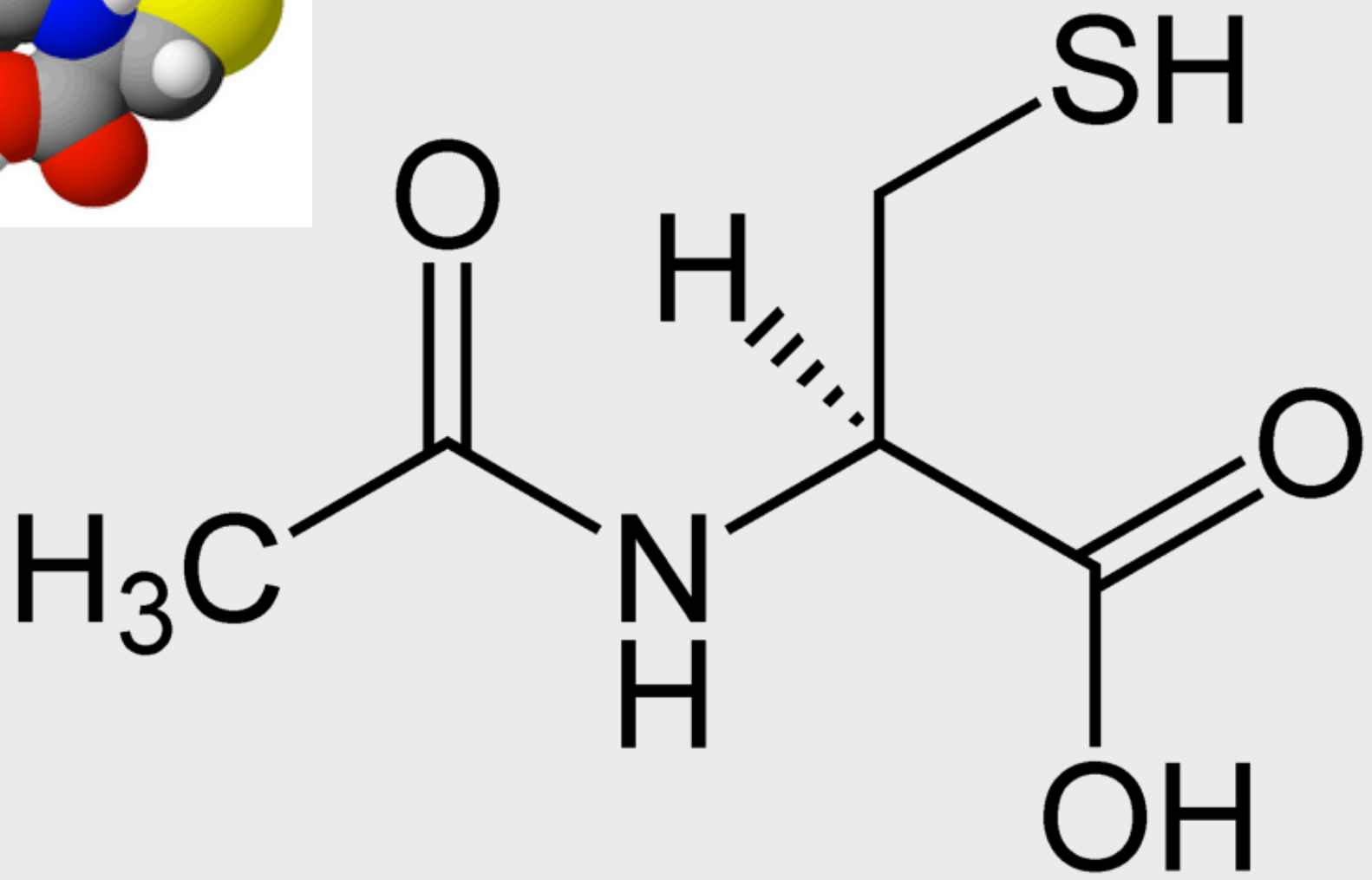
  High oxidative stress causes increased DNA damage, increased hematopoietic stem cell (HSC) senescence and a decreased HSC pool; thereby leading to bone marrow failure.

  In vivo and in vitro studies have demonstrated the ability of NAC to reduce DNA damage, reduce HSC senescence and augment reconstitution ability.

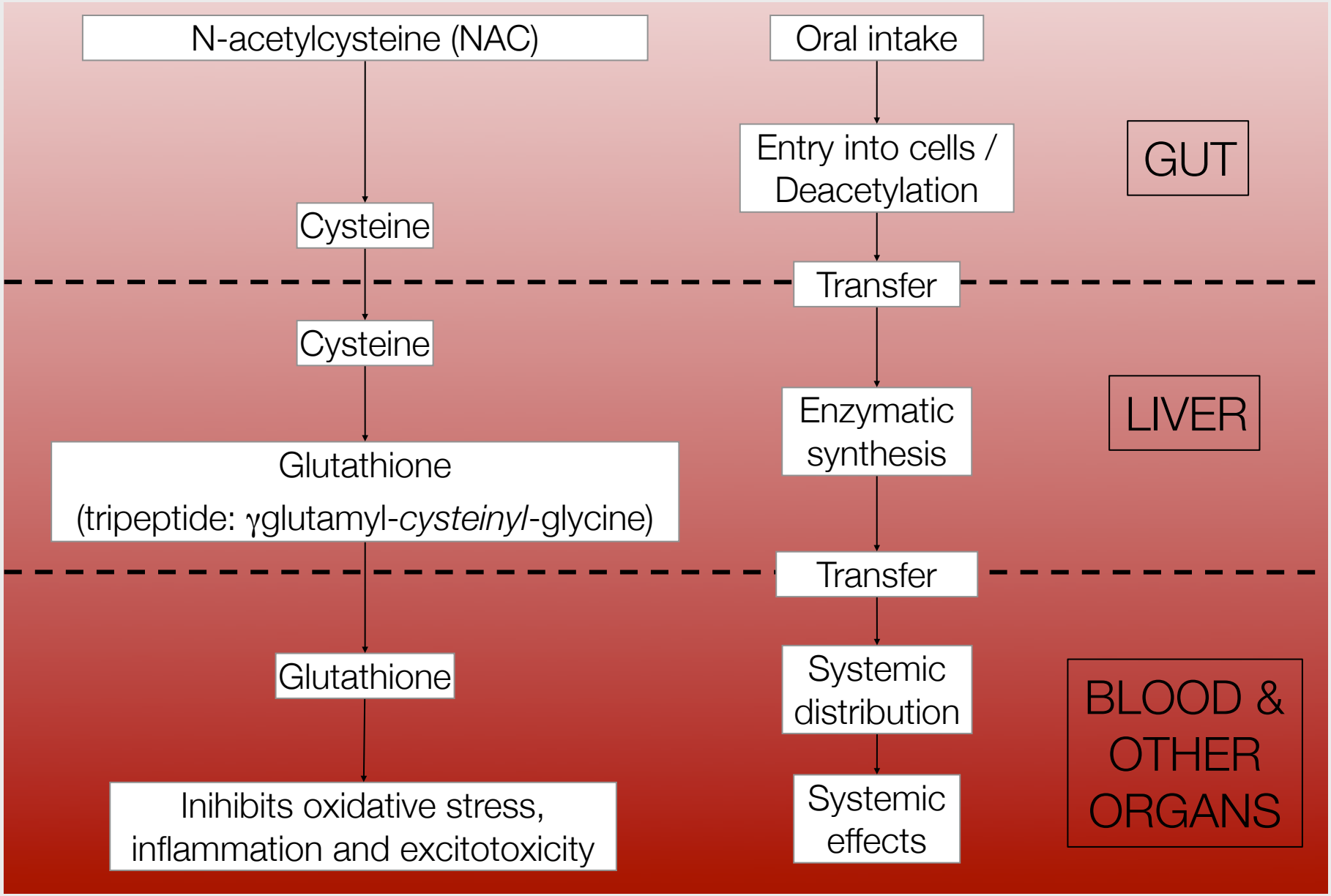
- **Hypothesis**

  Treatment with oral NAC will be well-tolerated and may ameliorate DNA damage and oxidative stress, and progressive bone marrow failure in patients with FA.

What is NAC?



How does NAC work?



Prior clinical experience with NAC

- **NAC is safe when given orally, in high doses**

In our own prior phase 2 trials (cystic fibrosis: NCT00809094, autism: NCT00627705), only significant side effect was mild digestive upset in some patients (same for placebo and NAC)

- **NAC inhibits various pathological processes**

Oxidative stress: liver (acetaminophen), kidney (contrast agent)

Inflammation: cystic fibrosis

Excitability: autism, obsessive compulsive disorders

- **CAUTION: Formulation matters!**

NAC is not regulated as a drug; OTC formulations not reliable

Study aims

- **Aim 1: Evaluate the safety of high-dose oral NAC in FA**
  - Clinical examination, blood and BM count, compliance
  - Oncogenic potential (p53 expression in blood cells)

- **Aim 2: Evaluate the ability of high-dose oral NAC to improve hematopoiesis in FA**
  - Blood and BM count, frequency of transfusion
  - BM function (CD34+ cells)

- **Aim 3: Evaluate the effect of high-dose oral NAC on genme stability and oxidative stress in FA**
  - Chromosomal fragility, G2 phase block, DNA breaks
  - Cysteine, glutathione, aldehydes

Experimental plan

- **General scheme**
  - Prospective study
  - Phase I (10 patients): safety, compliance
  - Phase II (30 patients): safety, compliance and efficacy

- **Treatment**
  - All participants receive oral NAC, 0.9 g (fizzy tablet), 3 times a day for 6 months

- **Study sites**
  - Primary: Sick Kids (Toronto), Indiana Univ (Indianapolis), Univ Minnesota (Minneapolis), Gaslini Institute (Genoa)
  - Secondary: Open to other sites

Target population

Inclusion criteria

- Diagnosis of Fanconi Anemia: *all complementation groups**
- Cytopenia (mild to severe, single or bi or trilineage) as defined by Hb ≤ 12 and/or WBC ≤ 3.5 $x10^9$/l and/or Platelets ≤150 $x10^9$/l
- Bone marrow analysis demonstrating normal karyotype
- Age ≥ 2 and ≤ 45 years at the time of enrolment
- Subject and/or guardian able to give informed consent

*modified in July 2013 after FA Family Meeting

Exclusion criteria

- Other investigational drugs or change in treatments within 90 d prior to screening or during trial
- Antioxidants and GSH prodrugs within 30 days prior to trial
- Known allergy to any constituent of the investigational drug
- History or evidence of malignancy / myelodysplasia
- History of hepatitis B or C or HIV
- Patients post- or anticipated to undergo stem cell transplantation
- Pregnant females

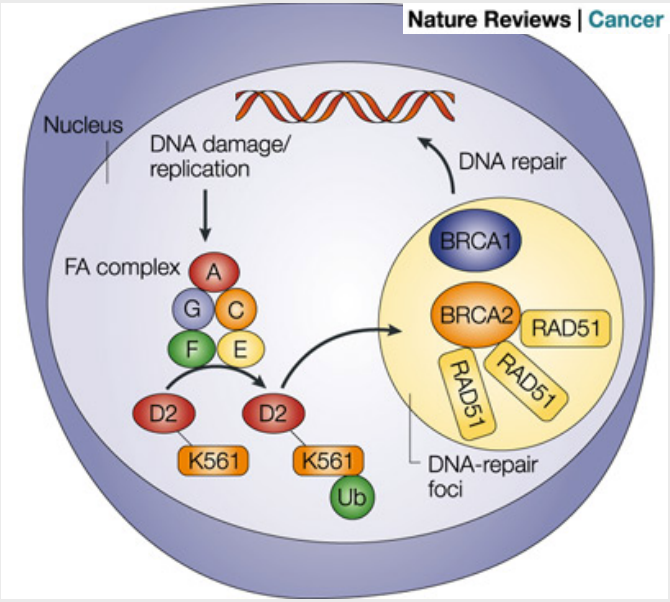
Experimental schedule

Timepoints	Clinical tests	Research samples / tests
MAIN: - Pre-treatment (within 1 week) - 6 month (end of trial) - 12 month (optional extension)	- History, physical examination - Complete blood count with reticulocyte hemoglobin F - Blood chemistry (Alb., Alk Phos, ALT, AST, Bil., Ca, Cl, Creat., Na, K, P, Prot., Urea) - Bone marrow testing (aspirate, trephine biopsy for cytology, morphology, iron stain, cytogenetics)	- Blood: DNA damage and chromosome fragility, p53 - Blood and plasma: Oxidative stress (Cys / Cys-Cys, GSH / GSSG, Glu, Aldehydes) - Bone marrow aspirate: DNA damage, Clonogenic assay, - Mouth wash and urine: Banking
INTERMEDIATE: - 6, 12, 18 weeks	- History, physical examination - Complete blood count	- N/A

Summary

Oxidative stress is a hallmark of FA

- FA is linked to ↑ oxidative stress / aldehydes
- FA is linked to ↑ DNA instability, as well as ↓ hematological function



NAC as a systemic treatment in FA: a "low-hanging fruit"

- Advantages: safe, cheap, repurposed (fast path to FDA approval)
- Expected outcomes: ↓ oxidative stress, ↓ DNA instability, ↑ hematological function

Proposed phase I / II trial

- Safety of NAC (high-dose: 2.7 g per day, duration: 6 months, N=40)
- Efficacy of NAC (DNA stability, hematological function, oxidative stress)