The preclinical efficacy of a novel telomerase inhibitor, imetelstat, in AML: A randomized trial in patient-derived xenografts

Claudia Bruedigam, Ph.D
Gordon and Jessie Gilmour Leukaemia Research Laboratory
Headed by A/Professor Steven Lane
Telomerase is activated to maintain the long-term replicative potential in most cancers including AML

- Telomerase is overexpressed in most AML
  Roth et al., Leukemia 2003

- AML oncogenes activate telomerase
  Gessner et al., Leukemia 2010

- LSC have shortened telomeres and increased telomerase activity
  Drummond et al., Leukemia 2005, Bernard et al., Leukemia 2009

- Genetic depletion of telomerase eradicates LSC upon enforced replication via cell cycle arrest and apoptosis
  Bruedigam et al., Cell Stem Cell 2014
Imetelstat (JNJ-63935937) is a competitive inhibitor of telomerase activity

- Imetelstat is a covalently lipated 13-mer oligonucleotide that binds the RNA template of telomerase
  *Herbert et al., Oncogene 2005*

- Imetelstat induced molecular and complete hematological responses in essential thrombocythemia (89%)
  *Baerlocher et al., NEJM 2015*

- Imetelstat showed efficacy in myelofibrosis (complete or partial remission in 21%)
  *Tefferi et al., NEJM 2015*

- Phase II / III trial to evaluate imetelstat in low or intermediate-1 risk myelodysplastic syndrome
  *NCT02598661*
Generating an AML patient-derived xenograft inventory

Primary AML patient sample

Sublethal irradiation (2.8 Gy)

Ficoll separation

CD3 depletion

NSGS

Engraftment

AML symptoms

Blast morphology
(Wright-Giemsa: peripheral blood)

Engraftment
(Peripheral blood donor chimerism)

Donor chimerism [%]

Weeks post-transplant

Survival

Individual samples tested (#): 35
Engraftment success rate (%): 72
Median survival (days): 220

AML marker expression
(Bone marrow and spleen)

hCD45

mCd45.1

hGPR56

hCD34

hCD38

hCD33

hCD45

Percent survival

Days post-transplant

hCD45

hCD45

hCD45

hCD45
Preclinical testing of imetelstat in AML PDX

Age at diagnosis

Gender

# AML patient samples

Primary or serial AML patient sample

Sublethal irradiation (2.8 Gy)

NSGS

(CD3 depletion)

(Check peripheral blood donor chimerism)

Randomize

Imetelstat (15 mg / kg bw) or PBS tiw ip

Engraftment

AML symptoms

ELN prognosis

(Doehner et al., Blood 2010
Alpermann et al., Blood 2011)
Imetelstat prolongs overall survival in AML PDX

Median survival:

PBS: 83
Imetelstat: 153

p < 0.0001
Imetelstat suppresses AML expansion in 14 out of 15 PDX

Overall survival

Percent survival

0 100

50

0

Days post-start of treatment

PBS

Imetelstat

Median survival:

PBS: 83

Imetelstat: 153

p < 0.0001

AML expansion

Peripheral blood donor chimerism [%]

Days post-start of treatment

Median survival:

PBS: 83

Imetelstat: 153

p < 0.0001
AML PDX can be separated into two groups with distinct response to imetelstat therapy

Sustained responders

Poor responders

ELN prognosis
(Sustained responders)

ELN prognosis
(Poor responders)
Next generation sequencing reveals baseline mutations in AML patient samples

HemePACT assay in collaboration with Stanley Chun-Wei Lee and Omar Abdel-Wahab, MSKCC
The identity and distribution of mutations in selected PDX reflects larger AML cohorts

Papaemmanuil et al., NEJM 2016

HemePACT assay in collaboration with Stanley Chun-Wei Lee and Omar Abdel-Wahab, MSKCC
Imetelstat response is correlated with a distinct mutational landscape

Mutations occurring in both sustained and poor responders

- Sustained responders
  - NPM1
  - DNMT3A
  - IDH2
  - KRAS
  - EZH2
  - PTPN11
  - SRSF2

- Poor responders
  - NPM1
  - DNMT3A
  - IDH2
  - KRAS
  - EZH2
  - PTPN11
  - SRSF2
Imetelstat response is correlated with a distinct mutational landscape

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Sustained responders

Poor responders
Imetelstat response is correlated with a distinct mutational landscape

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| NPM1 | DNMT3A | IDH2 | KRAS | EZH2 | PTPN11 | SRSF2 | FLT3 | NRAS | TET2 | ARID1B | CD36 | ECT2L | JAK3 | KDM2B | MAGED1 | MSH2 | MSH3 | PARK2 | PTPRD | RAD54L | RUNX1 | SMIC1A | STAG1 | ZNF703 | ETV6 | EAF5CM | MKI67 | WT1 | APC | ASXL1 | AXL | BCR | BCORL1 | BCRA1 | BRD4 | CXCR4 | ESR1 | ETS1 | FANCF | PCLG2 | SMO | TLL2 | TRAF2 | U2AF1 |
|-------|--------|------|------|------|--------|-------|------|------|------|--------|------|-------|------|-------|---------|------|------|-------|-------|--------|-------|--------|-------|------|-------|-------|-----|-----|------|------|-----|------|------|------|------|------|-----|-----|-----|-----|
Gene ontology analysis of mutually exclusive mutations reveals distinct molecular pathways

Fold enrichment

Sustained responders
- postreplication repair
- double-strand break repair
- response to ionizing radiation
- DNA recombination
- chromosome segregation
- response to radiation
- DNA repair
- response to DNA damage stimulus
- response to abiotic stimulus
- DNA metabolic process
- cell cycle process
- cellular response to stress
- apoptosis
- programmed cell death
- cell death
- death
- cell cycle

Fold enrichment

Poor responders
- regulation of myeloid cell differentiation
- positive regulation of cell differentiation
- pattern specification process
- Pathways in cancer
- immune system development
- positive regulation of developmental process
- chromosome organization
- regulation of programmed cell death
- regulation of cell death
Gene ontology analysis of mutually exclusive mutations reveals distinct molecular pathways

1. DNA repair
Gene ontology analysis of mutually exclusive mutations reveals distinct molecular pathways

1. DNA repair
2. Cell cycle

- Sustained responders:
  - DNA repair
  - Double-strand break repair
  - Response to ionizing radiation
  - DNA recombination
  - Chromosome segregation
  - Response to radiation
  - DNA repair
  - Response to DNA damage stimulus
  - Response to abiotic stimulus
  - DNA metabolic process
  - Cell cycle process
  - Cellular response to stress
  - Apoptosis
  - Programmed cell death
  - Cell death
  - Cell cycle

- Poor responders:
  - Regulation of myeloid cell differentiation
  - Positive regulation of myeloid cell differentiation
  - Pattern specification process
  - Pathways in cancer
  - Immune system development
  - Positive regulation of developmental process
  - Chromosome organization
  - Regulation of programmed cell death
  - Regulation of cell death
Gene ontology analysis of mutually exclusive mutations reveals distinct molecular pathways

1. DNA repair
2. Cell cycle
3. Development and differentiation
Gene ontology analysis of mutually exclusive mutations reveals distinct molecular pathways

1. DNA repair
2. Cell cycle

3. Development and differentiation

4. Pathways in cancer
Imetelstat induces DNA damage and loss of quiescence in LSC *in vivo*

AML patient sample

Sublethal irradiation (2.8 Gy)

(Check peripheral blood donor chimerism)

Randomize

Imetelstat (15 mg / kg bw) or PBS tiw ip

Endpoint analysis at disease onset of PBS group

**αH2AX**

**LSC in G0 [%]**

- **PBS**
- **Imetelstat**

AML patient sample

- **NSGS**

Sublethal irradiation (2.8 Gy)

(Check peripheral blood donor chimerism)

Randomize

Imetelstat (15 mg / kg bw) or PBS tiw ip

Endpoint analysis at disease onset of PBS group
Modelling normal human hematopoiesis

Cord blood donor sample

Sublethal irradiation (2.8 Gy) → CD34 enrichment

NSG

Imetelstat (15 mg / kg bw) or PBS tiw ip

10 weeks

Human hematopoiesis phenotype endpoint analysis
Imetelstat primarily depletes B lymphocytes

Cord blood donor sample

Sublethal irradiation (2.8 Gy) → CD34 enrichment → NSG

Imetelstat (15 mg / kg bw) or PBS tiw ip → 10 weeks → Human hematopoiesis phenotype endpoint analysis

Donor chimerism (Spleen)

B cells (Spleen)

Myeloid cells (Spleen)

Donor 1: PBS
Donor 1: Imetelstat
Donor 2: PBS
Donor 2: Imetelstat

$\text{Donor chimerism [%]}$

$\text{CD19+ [% of viable CD45+]}$

$\text{CD33+ [% of viable CD45+]}$

* $p = 0.08$

** $p < 0.0001$

### CD19+

- Donor 1: PBS
- Donor 1: Imetelstat
- Donor 2: PBS
- Donor 2: Imetelstat

- $p = 0.00667$

### CD33+

- Donor 1: PBS
- Donor 1: Imetelstat
- Donor 2: PBS
- Donor 2: Imetelstat

- $p = 0.0667$
Human cord blood - derived stem cells are preserved during imetelstat treatment

Cord blood donor sample

Sublethal irradiation (2.8 Gy)

CD34 enrichment

NSG

Imetelstat (15 mg / kg bw) or PBS tiw ip

10 weeks

Human hematopoiesis phenotype endpoint analysis
Summary: Preclinical efficacy of imetelstat in AML PDX

- Imetelstat is effective in a subgroup (60%) of AML patient samples

- Imetelstat prevents expansion and prolongs overall survival in AML PDX (PBS: 83 days; Imetelstat: 153 days post-start of treatment)

- Sustained responses to imetelstat are correlated with favorable cytogenetics, mutational profiles of DNA damage and activation of DNA damage response pathways

- This study has generated preclinical data to inform clinical trials and provide a precision approach to targeted therapies in patients with AML
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