**IDH1 and IDH2 mutations as friends and foes in human cancer**

Remco J. Molenaar, MD PhD
Post-doctoral research fellow
Resident Internal Medicine

Cleveland Clinic

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Isocitrate dehydrogenase (IDH) mutations in cancer
110,000 cancer cases
5,000 IDH-mutated cancers
*IDH* mutations associate with longer overall survival

Molenaar *et al*, Neuro Oncology 2014
IDH in cellular metabolism

Molenaar et al., BMJ Open 2017
Wild-type IDH

NADP$^+$ + isocitrate $\rightarrow$ NADPH

NADP$^+$ + alpha-ketoglutarate $\rightarrow$ NADPH

Molenaar et al, BBA Rev Cancer 2014; Oncogene 2018
Mutated IDH

\[
\text{Mutated IDH} \quad \text{NADP}^+ + \text{isocitrate} \rightarrow \text{alpha-ketoglutarate} \quad \text{NADPH}
\]

\[
\text{D-2-hydroxyglutarate} \quad \text{NADP}^+ \rightarrow \text{mutant} \quad \text{NADPH}
\]

Molenaar et al, BBA Rev Cancer 2014; Oncogene 2018
**IDH1/2 mutations are early genetic events in glioblastoma**

Bleeker, Molenaar et al, J Neurooncology 2012
IDH mutations associate with longer overall survival

Association or causation?

- Intrinsic effect (slower tumor growth)
- Extrinsic effect (sensitization for therapy)

Molenaar et al, Neuro Oncology 2014
Increased DNA damage

$D-2HG$↑

less DNA repair

NADPH↓

more reactive oxygen species

Molenaar et al, BBA Rev Cancer 2014; Lancet 2014; Cancer Research 2015; Clinical Cancer Research 2018
Increased DNA damage

\[ D-2HG \uparrow \]

+ cytotoxic therapy

\[ \text{NADPH} \downarrow \]

= less DNA repair

more reactive oxygen species

Molenaar et al, BBA Rev Cancer 2014; Lancet 2014; Cancer Research 2015; Clinical Cancer Research 2018; Oncogene 2018
Increased DNA damage

\[ D-2HG \uparrow \]

+ cytotoxic therapy

\[ \downarrow \text{NADPH} \]

= less DNA repair

more reactive oxygen species

Molenaar et al, BBA Rev Cancer 2014; Lancet 2014; Cancer Research 2015; Clinical Cancer Research 2018; Oncogene 2018
Increased DNA damage

\[ D-2HG \] + cytotoxic therapy

\[ \text{NADPH} \]

\( \text{Increased cytotoxic therapy responses} \)

Molenaar et al, BBA Rev Cancer 2014; Lancet 2014; Cancer Research 2015; Clinical Cancer Research 2018; Oncogene 2018
HCT116 \( IDH1^{R132H} \) knock-in as a mechanistic model

**IDH1-mutated cell**

**HCT116 knock-in (\( IDH1^{WT/R132H} \))**

**IDH1 wild-type cell**

**HCT116 parental (\( IDH1^{WT/WT} \))**

Molenaar et al, Cancer Research 2015; Clinical Cancer Research 2018
IDH$_{1}^{R132H}$ radiosensitizes HCT116 cells

Molenaar et al, Cancer Research 2015; Clinical Cancer Research 2018
D-2HG radiosensitizes HCT116 cells

Molenaar et al, Cancer Research 2015
Measuring enzyme kinetics *in situ*
IDH1$^{R132H}$ decreases NADPH production capacity.

$\text{IDH1}^{\text{WT/WT}}$  $\text{IDH1}^{\text{WT/R132H}}$

Absorbance

$0.05$  $0.10$  $0.15$ $0$  $1$  $2$  $3$  $4$  $5$
mM isocitrate

$\text{IDH1}^{\text{WT/WT}}_{\text{HCT116}}$  $\text{IDH1}^{\text{WT/R132H}}_{\text{HCT116}}$

Molenaar et al, Cancer Research 2015
D-2HG decreases NADPH production capacity

Molenaar et al, Cancer Research 2015
$D$-2HG decreases NADPH production capacity

Molenaar et al, Cancer Research 2015
**IDH1** mutations increase ROS
N-acetyl cysteine equalizes radiosensitivity of HCT116 cells
**IDH1** mutations increase DNA damage levels

Molenaar et al, Cancer Research 2015
**IDH1** therapy sensitivity cascade

- **IDH1/2 mutation**
- **D-2HG accumulation**
- **SOLID ONC**
  - Decrease in IDH1/2\(^{WT}\)-mediated NADPH production capacity
  - Decreased reducing power
    - ROS scavengers (e.g. NAC)
- **Increased DNA damage**
- **Increased therapy sensitivity**

Molenaar *et al*, Cancer Research 2015; Clinical Cancer Research 2018; Oncogene 2018
Pharma’s incentive…

• Hotspot mutations
• Catalytic site
• Addiction to D-2HG
• It’s care, it’s not a cure
IDH-mutant inhibitors
IDH-mutant inhibitors

- Monotherapy
- ~40% responses
- 2nd line for AML
- In clinical trials for glioma, cholangiocarcinoma, chondrosarcoma…
An IDH1-mutant inhibitor restores NADPH production
An IDH1-mutant inhibitor restores NADPH production.
IDH1 mutant inhibitors decrease ROS
IDH-mutant inhibitors
IDH mutations and survival

Molenaar et al, Neuro Oncology 2014
Increased DNA damage

\[ D-2HG \uparrow \]

+ cytotoxic therapy

\[ \Downarrow \] NADPH

\[ \text{Increased cytotoxic therapy responses} \]
IDH mutations and survival

Molenaar et al, Neuro Oncology 2014
**IDH-mutated cells, radiation and an IDH-mutant inhibitor**

Molenaar et al, Cancer Research 2015
No effect in *IDH* wild-type

Molenaar et al, Cancer Research 2015
IDH1 therapy sensitivity cascade

IDH1/2 mutation

IDH1/2\textsuperscript{MUT} inhibitors

D-2HG accumulation

Decrease in IDH1/2\textsuperscript{WT}-mediated NADPH production capacity

Decreased reducing power

Increased DNA damage

Increased therapy sensitivity

SOLID ONC

ROS scavengers (e.g. NAC)

Molenaar et al, Cancer Research 2015; Clinical Cancer Research 2018
An IDH1-mutant inhibitor cannot protect in the presence of D-2HG
**IDH1** mutations increase DNA damage levels

Molenaar et al, Cancer Research 2015
**IDH1 therapy sensitivity cascade**

- **IDH1/2 mutation**
- **D-2HG accumulation**
- **Decrease in IDH1/2\(^{WT}\) mediated NADPH production capacity**
- **Decreased reducing power**
- **Increased DNA damage**
- **Increased therapy sensitivity**
- **SOLID ONC** (IDH1/2\(^{MUT}\) inhibitors)

**Additional Notes:**
- ROS scavengers (e.g. NAC)

Reference:
Molenaar *et al*, Cancer Research 2015; Clinical Cancer Research 2018
Conclusions (1)

- *IDH* mutations sensitise cancer cells to cytotoxic therapy

- Elucidation of the underlying mechanism

- IDH-mutant inhibitors protect *IDH*-mutated cancer cells against cytotoxic therapy

- Rational design of clinical trials

Molenaar *et al*, Cancer Research 2015; BMJ Open 2017; Clinical Cancer Research 2018
The association between our research and Agios stock prices
The association between our research and Agios stock prices
IDH1/2 mutations sensitize AML cells to daunorubicin

Molenaar et al, Clinical Cancer Research 2018
An IDH1-mutant inhibitor protects against daunorubicin
An IDH2-mutant inhibitor protects against daunorubicin

Molenaar et al, Clinical Cancer Research 2018
IDH1/2-mutant inhibitors protect against daunorubicin

Molenaar et al, Clinical Cancer Research 2018
IDH1/2 mutation

D-2HG accumulation

IDH1/2^{MUT} inhibitors

Decrease in IDH1/2^{WT}-mediated NADPH production capacity

Decreased reducing power

Increased DNA damage

Increased therapy sensitivity

SOLID ONC

ROS scavengers (e.g. NAC)

Molenaar et al, Cancer Research 2015; Clinical Cancer Research 2018
NADPH production is reduced in \textit{IDH1/2}-mutated AML

Molenaar et al, Clinical Cancer Research 2018
IDH1/2-mutant inhibitors restore NADPH production

C

Relative activity (a.u.)

- + - - - - -

AGI-5198
AGI-6780
D-2HG

IDH1\textsuperscript{MUT}
IDH2\textsuperscript{MUT}
IDH1/2\textsuperscript{WT}

Molenaar et al, Clinical Cancer Research 2018
Redox states are unchanged in \textit{IDH1/2}-mutated AML

\begin{itemize}
\item \textit{IDH1/2}^{WT}
\item \textit{IDH1}^{MUT}
\item \textit{IDH2}^{MUT}
\end{itemize}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart}
\caption{Graph showing NADP⁺:NADPH ratio, GSSG:GSH ratio, and ROS levels for \textit{IDH1/2}^{WT}, \textit{IDH1}^{MUT}, and \textit{IDH2}^{MUT}.
Control, 50 nM DAU, 2 Gy IR.}
\end{figure}
IDH1/2 mutation → D-2HG accumulation

IDH1/2^{MUT} inhibitors

AML

???

Decrease in IDH1/2^{WT}-mediated NADPH production capacity

Decreased reducing power

Increased DNA damage

Increased therapy sensitivity

ROS scavengers (e.g. NAC)

Molenaar et al, Cancer Research 2015; Clinical Cancer Research 2018
IDH1/2 mutations reduce ATM expression
IDH1/2 mutations increase DNA damage in AML

Molenaar et al, Clinical Cancer Research 2018
ATM is responsible for the sensitization to daunorubicin

![Graph showing clonogenic fraction vs. daunorubicin concentration for different conditions.]

- **IDH1/2^{WT}**
- **IDH1/2^{WT} + D-2HG**
- **IDH1/2^{WT} + siRNA**
- **IDH1/2^{WT} + siRNA + D-2HG**

Molenaar et al, Clinical Cancer Research 2018
ATM is responsible for the sensitization to daunorubicin
ATM is responsible for the sensitization to daunorubicin

Molenaar et al, Clinical Cancer Research 2018
**IDH1/2 therapy sensitivity cascade**

1. **IDH1/2 mutation**
2. **D-2HG accumulation**
   - **IDH1/2\textsuperscript{MUT} inhibitors**
     - Reduced ATM expression
     - Decreased DNA damage response
   - Increased DNA damage
     - Increased therapy sensitivity
3. **Decrease in IDH1/2\textsuperscript{WT}-mediated NADPH production capacity**
   - Decreased reducing power
   - ROS scavengers (e.g., NAC)

**AML**

**SOLID ONC**

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Molenaar et al., Cancer Research 2015; Clinical Cancer Research 2018
Conclusions (2)

- *IDH1/2* mutations sensitize solid tumor and AML cells to cytotoxic therapy

- Elucidation of the underlying mechanism
  - NADPH in solid tumor cells
  - ATM suppression in AML cells

- *IDH1/2*-mutant inhibitors protect *IDH1/2*-mutated cancer cells against cytotoxic therapy

Molenaar *et al*, Cancer Research 2015; BMJ Open 2017; Clinical Cancer Research 2018
IDH1/2 mutations sensitize AML cells to PARP inhibition
IDH1/2-mutant inhibitors protect against PARP inhibition

Molenaar et al, Clinical Cancer Research 2018
IDH1/2-mutant inhibitors protect against PARP inhibition.

Molenaar et al., Clinical Cancer Research 2018
IDH1/2-mutant inhibitors protect against PARP inhibition

Molenaar et al, Clinical Cancer Research 2018
IDH1/2-mutant inhibitors protect against PARP inhibition

Molenaar et al, Clinical Cancer Research 2018
Conclusions (3)

- *IDH1/2* mutations sensitize AML cells to PARP inhibitors

- Promising therapy for *IDH1/2*-mutated AML:
  - PARP inhibitor monotherapy
  - PARP inhibitor + cytotoxic agent(s)

Molenaar *et al*, Cancer Research 2015; BMJ Open 2017; Clinical Cancer Research 2018
IDH in cellular metabolism

Molenaar et al, BMJ Open 2017
IDH in cellular metabolism

Molenaar et al, BMJ Open 2017
IDH in cellular metabolism

Molenaar et al, BMJ Open 2017
Metformin and chloroquine for IDH1/2-mutated tumors

Screening:
- D-2HG test (MS)
- NGS of tumor DNA
- IHC of tumor tissue

Start of study:
- D-2HG test (MS + MRS)
- NGS of liquid biopsy

Every 4 weeks:
- D-2HG test (MS)
- NGS of liquid biopsy
- Pharmacokinetics test

End of study:
- D-2HG test (MS + MRS)
- NGS of liquid biopsy

Metformin starting dose 500 mg q.d. (days 1-5)

Metformin escalation dose (day 6 and later; Table 2)

Chloroquine study dose 200 mg q.d. (week 2 and later)

Molenaar et al, BMJ Open 2017
Metformin and chloroquine for IDH1/2-mutated tumors

Screening:
- D-2HG test (MS)
- NGS of tumor DNA
- IHC of tumor tissue

Metformin starting dose 500 mg q.d. (days 1-5)

Metformin escalation dose (day 6 and later; Table 2)

Chloroquine starting dose 300 mg q.d. (weeks 2-3)

Chloroquine maintenance dose 200 mg q.d. (week 4 and later)

Every 4 weeks:
- D-2HG test (MS)
- NGS of liquid biopsy
- Pharmacokinetics test

Start standard therapy (not part of this study)

Start of study:
- D-2HG test (MRS)
- NGS of liquid biopsy

End of study:
- D-2HG test (MS + MRS)
- NGS of liquid biopsy

Diagnosis

Molenaar et al, BMJ Open 2017
Conclusions (4)

Metformin → Changes in metabolism → \( \text{D-2-hydroxyglutarate} \) → Less DNA repair → PARP inhibitor

\[ \text{isocitrate} \quad \text{NADP}^+ \quad \text{wild-type} \quad \text{mutant} \quad \text{NADPH} \quad \text{alpha-ketoglutarate} \quad \text{NADP}^+ \quad \text{NADPH} \]

Molenaar et al, BBA Rev Cancer 2014; BMJ Open 2017; Clinical Cancer Research 2018
All of our patients and families