IMMUNOMODULATORY ACTIVITY OF SGI-110, A SECOND GENERATION HYPOMETHYLATING AGENT

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DNA Methylation as a Therapeutic Target

• DNA methylation is abnormal in most cancers and affects the expression of key genes and pathways
• DNA methylation and epigenetic readers and writers are often mutated in cancer
  – In leukemias: DNMT3a, TET2, EZH2, ASXL1, MLL1-3, CBP etc.
• The cancer phenotype can be reversed by DNA methylation reprogramming
• DMNT inhibitors or Hypomethylating Agents (HMAs) demonstrated efficacy in the treatment of MDS and AML
Cytosine Analogues as HMAs

Cytosine 5-methylcytosine 5-aza-cytidine 5-aza-2'-deoxy-cytidine

Azacitidine (2004)\(^1\)
Decitabine (2006)\(^1\) (2012)\(^2\)

\(^1\)Year approved by FDA for MDS treatment
\(^2\)Year approved by EMA for AML treatment

IMMUNOMODULATORY ACTIVITY OF DECITABINE

**Pre-clinical**

- Induction/up-regulation of CTA expression in tumor cells of different histotype (Coral, Clin Cancer Res 2002)
- Up-regulation of the expression of HLA class I antigens and co-stimulatory molecules in tumor cells of different histotype (Fonsatti, Clin Cancer Res 2007)
- Increased recognition of cancer cells treated with decitabine by TAA-specific CTL (Sigalotti, Cancer Res 2004)
- Persistent induction/up-regulation of CTA expression in tumor xenografts (Coral, J Cell Physiol 2006)
  - Generation of circulating anti-CTA antibodies in mice injected with decitabine-treated human melanoma cells (Coral, J Cell Physiol 2006)

**Clinical**

- Induction of CTA expression in AML and MDS patients (Sigalotti et al, Blood 2003)
- Post-treatment generation of circulating anti-CTA antibodies in patients with thoracic malignancies (Schrump, Clin Cancer Res 2006)
- Complete remission following decitabine/dendritic cell vaccine in a case of relapsed neuroblastoma (Krishnadas, Pediatrics, 2012)
New DNMT Inhibitor: SGI-110

- Decitabine is rapidly eliminated by Cytidine Deaminase, limiting drug exposure time to cancer cells \textit{in vivo}

- SGI-110 is a Dinucleotide of Decitabine and Deoxyguanosine that increases the \textit{in vivo} exposure of decitabine by protecting it from deamination
SGI-110 Modulates CTA Expression and Methylation in Cancer Cells

SGI-110 induces the demethylation of CTA promoters and induces their expression
Recognition of SGI-110-treated Mel 275 melanoma cells by gp100-specific CTL

![Graph showing % of cytotoxicity vs E/T ratio for Mel 275 cells treated with SGI-110 and control conditions.](image)

- **SGI-110**
- **Ctrl**

![Bar graph comparing % of cytotoxicity for different cell lines and treatments.](image)

- **Mel 275**
- **Mel 40 K562**

- **SGI-110**
- **α-HLA class I**
- **α-ICAM-I**
SGI-110 Modulates CTA Expression and Immune Phenotype of Melanoma Xenografts

NY-ESO-1 Induction in Mel313 xenografts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NY-ESO-1 /β-actin mol</th>
</tr>
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<tbody>
<tr>
<td>Ctrl 5 days</td>
<td>0.00E+00</td>
</tr>
<tr>
<td>3mg/kg weekly</td>
<td>5.00E-04</td>
</tr>
<tr>
<td>6.1mg/kg weekly</td>
<td>1.00E-03</td>
</tr>
<tr>
<td>12.2mg/kg weekly</td>
<td>1.00E-03</td>
</tr>
<tr>
<td>24.4mg/kg weekly</td>
<td>1.00E-03</td>
</tr>
</tbody>
</table>

HLA Class I and co-stimulatory molecules induction in Mel195 xenografts

Tolerated doses and schedules of SQ SGI-110 induces CTAs, HLA class I antigens, HLA-A2 alleles, and the co-stimulatory molecules LFA-3 and ICAM-1 in melanoma xenografts
SGI-110-01 Phase 1/2 Clinical Trial Design

**Part A**
**Dose Escalation (78 pts)**

- Regimen 1
  - Daily SC Days 1–5 of a 28-day course

- Regimen 2
  - Weekly SC x 3 of a 28-day course

PK-PD guided dose escalation

**Part B**
**Dose Expansion (~160 pts)**

- Safety, Efficacy, PK – PD Assessments
  - $C_{max}$, AUC, Global Hypomethylation

- 60 mg/m² dailyx5
- 90 mg/m² dailyx5

3 Groups: Relapsed/refractory AML; Treatment naïve elderly AML; Treatment naïve MDS
PK of decitabine delivered by SGI-110 SQ injection

Compared to Dacogen IV (DAC IV):
• Doubled exposure window to decitabine (8+ hrs vs. 3-4 hrs)
• Up to 4-fold longer half life of decitabine (1.5-2.5 hrs vs. 35 minutes)
• Cmax less than half of decitabine
LINE1 Demethylation by Cohort

**Daily x 5**
- 3 mg/m² (n=4)
- 9 mg/m² (n=4)
- 18 mg/m² (n=3)
- 36 mg/m² (n=5)
- 60 mg/m² (n=5)
- 90 mg/m² (n=4)
- 125 mg/m² (n=10)

**Weekly x 3**
- 6 mg/m² (n=5)
- 18 mg/m² (n=3)
- 36 mg/m² (n=6)
- 60 mg/m² (n=5)
- 90 mg/m² (n=7)
- 125 mg/m² (n=4)

**BED**: 60 mg/m² daily x 5

The BED defined as the smallest dose that achieves a maximum global hypomethylation in at least three successive dose levels.
AML Responses correlated with demethylation extent

<table>
<thead>
<tr>
<th>LINE1 Demethylation</th>
<th>Number Treated¹</th>
<th>Responders (CR/CRi/CRp)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10%</td>
<td>31</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>≥ 10%</td>
<td>19</td>
<td>5</td>
<td>26%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>5</td>
<td>10%</td>
</tr>
</tbody>
</table>

¹ All 50 r/r AML patients with LINE1 data

5 responses in MDS patients with prior HMA treatment
5 responses in AML patients regardless of prior HMA treatment
EPIGENETIC MODULATION OF CTA IN BLOOD SAMPLES FROM PATIENTS ENROLLED IN STUDY SGI-110-01
NY-ESO-1 Promoter Demethylation after SGI-110 in AML and MDS patients

- SGI-110 induces a dose-dependent demethylation of NY-ESO-1 promoter
- Similar extent of demethylation observed also for MAGE-A1 promoter
NY-ESO-1 Induction (cut-off ≥ 1E-05) after SGI-110 in AML and MDS patients

- NY-ESO-1 transcript was induced in 9 of 15 evaluable patients treated at SGI-110 BED
- 4 and 5 of the 15 patients induced also MAGE-A1 and -A3 respectively
Summary

• Excellent LINE1 hypomethylation induction with dailyx5; BED is 60 mg/m² dailyx5
• Well tolerated; most common AE’s were Injection site pain (mostly Grade 1) and myelosuppression (neutropenia/neutropenic fever; anemia; thrombocytopenia)
• Major responses were observed in relapsed/refractory AML when adequate hypomethylation achieved (regardless of regimen)
• SGI-110 reduced the constitutive methylation levels in promoters of NY-ESO-1 and MAGE-A1 in a dose-dependent manner
• The induction and/or up-regulation of NY-ESO-1, MAGE-A1, MAGE-A3 expression was observed in 9/15, 4/15 and 5/15 patients treated with SGI-110 biologically effective doses
• These immunomodulatory properties and its favorable PK/PD profile make SGI-110 an active agent to implement new and more effective combined chemo-immunotherapeutic approaches
Acknowledgements: Clinical Study SGI-110-01

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Immunomodulatory Activity of SGI-110

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