D35 Project

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Services, GeneDesign

THE INSTITUTE OF
MEDICAL SCIENCE,
THE UNIVERSITY OF TOKYO

Gene Design Inc.

DNDi
Drugs for Neglected Diseases initiative

Global Health Innovative Technology Fund
What is CpG DNA?

CpG DNA is a general term of immunostimulatory DNA containing unmethylated CG dinucleotides with certain flanking sequences (=CpG motifs).

Bacterial DNA contains these CpG motifs ~20 fold more than vertebrate (mammalian) DNA due to CpG methylation and CpG suppression in mammalian DNA.

Oligonucleotides containing the CpG motifs (CpG ODN) mimic the activity of bacterial DNA.
What are CpG Motifs?

CpG motifs (mouse) $Pu-Pu-C-G-Py-Py$  Immunostimulatory Activity

<table>
<thead>
<tr>
<th></th>
<th>Sequence</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CpG ODN</td>
<td>T-C-A-A-C-G-T-T-G-A</td>
<td>++++</td>
</tr>
<tr>
<td>CpG ODN</td>
<td>T-C-G-A-C-G-T-C-G-A</td>
<td>++++</td>
</tr>
<tr>
<td>Methyl CpG ODN</td>
<td>T-C-GA-$mC$-G-T-C-G-A</td>
<td>-</td>
</tr>
<tr>
<td>Non CpG ODN</td>
<td>T-C-T-C-C-G-G-A-G-A</td>
<td>-</td>
</tr>
</tbody>
</table>
Molecular Basis of TLR-Ligand Interaction

A

B

TLR + Ligand = Binding

Activation signal ↔ Conformational changes

Ishii KJ and Akira S Cell Host Microbe 2008
Therapeutic applications of CpG ODN

- **CpG ODN**
  - ↑ Innate immune activation (↑ pDC, B, Mφ ⇒ ↑ NK cell)
  - ↓ IgE, ↓ Eosinophilia
  - ↑ Th1, ↓ Th2

- **Pathogen**
  - IFNs, IL12, NO, Chemokines
  - NK killing, phagocytosis

- **Tumor**
  - IFNs, IL12, NO, Chemokines
  - NK killing, phagocytosis

- **Vaccine**
  - ↑ IgG2a, IFNγ, CTL

- **Allergen**
  - ↓ IgE, ↓ Eosinophilia

- **Anti-infection**
  - IgG2a, IFNγ, CTL

- **Anti-Cancer**
  - IFNs, IL12, NO, Chemokines
  - NK killing, phagocytosis

- **Anti-Allergen**
  - IFNs, IL12, NO, Chemokines
  - NK killing, phagocytosis

- **Th1 Adjuvant**
  - IFNs, IL12, NO, Chemokines
  - NK killing, phagocytosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Organism</th>
<th>Challenged Dose</th>
<th>% protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td><em>L. monocytogenes</em></td>
<td>$10^3$ LD50</td>
<td>0</td>
</tr>
<tr>
<td>CpG ODN</td>
<td><em>L. monocytogenes</em></td>
<td>$10^3$</td>
<td>100</td>
</tr>
<tr>
<td>Saline</td>
<td>Malaria sporozoites</td>
<td>$10^2$</td>
<td>0</td>
</tr>
<tr>
<td>CpG ODN</td>
<td>Malaria sporozoites</td>
<td>$10^2$</td>
<td>88</td>
</tr>
<tr>
<td>Saline</td>
<td>Ebola virus</td>
<td>$10^3$</td>
<td>0</td>
</tr>
<tr>
<td>CpG ODN</td>
<td>Ebola virus</td>
<td>$10^3$</td>
<td>50</td>
</tr>
<tr>
<td>Saline</td>
<td><em>P. aeruginosa</em></td>
<td>$10^2$</td>
<td>0</td>
</tr>
<tr>
<td>CpG ODN</td>
<td><em>P. aeruginosa</em></td>
<td>$10^2$</td>
<td>0</td>
</tr>
</tbody>
</table>

CpG ODN Improves Host Resistance to a Variety of Pathogens

CpG ODN (50ug) → Challenge (3 days) → Time

**CpG ODN**

24h  
2-3days  
1-3wks

**TLR9/MyD88**

**B**

**Mφ**

**IL-12**

**NK**

**Pathogens or cancer**

**cDC**

**IL-12**

**Thy1.2 DC**

**IL-12, IFNγ**

**IFNγ, NO**

**pDC**

*(IFNα = X)*

**CD40L**

**IL-15**

**pDC**

**IKDC**

Activation, IL-12, IFNα(?) , IFNγ

**Polyreactive IgM**

**Phagocytosis, NO?**

**NK killing, IFNγ**
CpG ODN acts as an immunoprophylaxis against variety of inf. Org.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Organism</th>
<th>% of protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td><em>L. monocytogenes</em></td>
<td>0 or 100</td>
</tr>
<tr>
<td>Mouse</td>
<td><em>F. tularensis</em></td>
<td>100</td>
</tr>
<tr>
<td>Mouse</td>
<td><em>M. tuberculosis</em></td>
<td>60–100</td>
</tr>
<tr>
<td>Chicken</td>
<td><em>E. Coli</em></td>
<td>90–100</td>
</tr>
<tr>
<td>Mouse</td>
<td><em>P. yoelii</em></td>
<td>80</td>
</tr>
<tr>
<td>Mouse</td>
<td><em>L. major</em></td>
<td>100</td>
</tr>
<tr>
<td>Rhesus macaque</td>
<td><em>L. major</em></td>
<td>0 (K) or 100 (D)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Friend virus</td>
<td>74</td>
</tr>
<tr>
<td>Mouse</td>
<td>HSV-2</td>
<td>50</td>
</tr>
<tr>
<td>Mouse</td>
<td>Ebola virus</td>
<td>50</td>
</tr>
<tr>
<td>Mouse</td>
<td>RML prion</td>
<td>100</td>
</tr>
</tbody>
</table>

Obstacles

• Optimal CpG motifs for mouse are not active in human immune system.
• Low efficacy of DNA vaccines are observed currently in human clinical trials (than that of mouse).
• TLR9 expression in immune cells is different between human and mouse.

→ Needs humanized CpG motifs
Humanization of CpG DNA (ODN): Distinct types of CpG

Mouse CpG
NN-Pu-Pu-CpG-Py-PyNN

K-Type CpG ODN (PS)
NNNTCGT/ANNN

D-type CpG ODN (mix)
GGnnnatcgatnnnnngggGG

B cell

mDC

pDC

B cell

MHC, CD40, CD86
IFNα, TNFα, chemokines

Proliferation
Polyclonal activation
IL-6, IgM, chemokines

mDC

APC function
CD8 T cell activation

NK

↑ IFNα

NK activity
IFNγ

Klinman DM et al Patented 1999,
Required parameters for activity:
- Minimum length 18 bp
- PO Backbone
- Unmethylated CpG
- Central hexameric motif
- Selfcomplementary regions flanking the hexamer
- 3’ Poly Gs

Required parameters for activity:
- Minimum length 12 bp
- PS backbone
- multiple CpG motifs
## Individual innate immune response modifiers induce distinct responses in human PBMC.

<table>
<thead>
<tr>
<th></th>
<th>MPL (TLR4)</th>
<th>K ODN (TLR9)</th>
<th>D35 ODN (TLR9)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>(B cells)</td>
</tr>
<tr>
<td>IgM</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>(B cells)</td>
</tr>
<tr>
<td>IL-10</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>(B cells)</td>
</tr>
<tr>
<td>TNFα</td>
<td>+++</td>
<td>+++</td>
<td>+/-</td>
<td>(Monocytes)</td>
</tr>
<tr>
<td>IL-6</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>(Monocytes)</td>
</tr>
<tr>
<td>IP-10</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>(Monocytes)</td>
</tr>
<tr>
<td>IFNα</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>(pDC)</td>
</tr>
<tr>
<td>IFNγ</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>(NK cells)</td>
</tr>
</tbody>
</table>
Humanization of CpG DNA (ODN): Distinct types of CpG

Mouse CpG
NN-Pu-Pu-CpG-Py-PyNN

B cell mDC pDC

K(B) -Type CpG ODN (PS)
NNNTCGT/ANNN

B cell

Proliferation
Polyclonal activation
IL-6, IgM, chemokines

mDC

APC function
CD8 T cell activation

pDC

P(DA)-type CpG ODN (mix)
GGnnnatcgatnnngggGG

Proliferation
Polyclonal activation
IFNα, TNFα, chemokines

MHC, CD40, CD86

NK activity
IFNγ

IFNα

NK

K3 is on the way to human

1) Malaria vaccine BK-SE36 (Prof. T Horii) with Humanized CpG ODN (K3) / Aluminum hydroxide) Ph-1a (Osaka, 2013), Ph-2 (Uganda (Ph1-b) now in Ph-2 in Burkina Faso (AMED/GHIT/EVI)

2) used in human clinical trial for cancer immunotherapy and soon for vaccine adjuvant against AMR, Pandemic Flu,
Humanization of CpG DNA (ODN): Distinct types of CpG

Mouse CpG
NN-Pu-Pu-CpG-Py-PyNN

K(B) -Type CpG ODN (PS)
NNNTCGT/ANNN

D(A)-type CpG ODN (mix)
GGnnnatcgatnnngggGG

B cell

mDC

pDC

Proliferation
Polyclonal activation
IL-6, IgM, chemokines

mDC

APC function
CD8 T cell activation

IFNα

NK

NK activity
IFNγ

B cell

pDC

IFNγ

MHC, CD40, CD86
IFNα, TNFα, chemokines

A novel nucleic acid-based immunomodulator D35 for treatment of Leishmaniasis
Experience in murine models of leishmaniasis:

- CpG ODN can cure mice of lethal *L. major* infection, even when treatment is delayed until day 20 (Zimmermann et al., J. Immunol., 1998)
- Systemic or local CpG therapy prevents death in mice with footpad *L. major* infection. (Walker et al., PNAS, 1999)

- Clinical study BCG improved outcome as treatment adjuvant in CL and PKDL
How about Monkey?
Macaque treated \textit{in vivo} with D35 (SC 0.5mg/kg on days 1, 3 & 7). 5 macaques per group.
Fold increase in gene expression in skin over time in macaques injected with D35 or L. major (note response in hours vs. days)

<table>
<thead>
<tr>
<th>gene</th>
<th>SALINE</th>
<th>D-ODN (hours)</th>
<th>Leishmania (days post infection)</th>
</tr>
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<tbody>
<tr>
<td>pDC</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CD209</td>
<td>0</td>
<td>6</td>
<td>24</td>
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<td>CD80</td>
<td>24</td>
<td>48</td>
<td>72</td>
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<td>CD83</td>
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<tr>
<td>CD86</td>
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<tr>
<td>Type I IFN</td>
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<tr>
<td>IFNa2</td>
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<tr>
<td>IFNB1</td>
<td>&gt;5000</td>
<td>50-100</td>
<td>100-500</td>
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<tr>
<td>TLR &amp; IRF</td>
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<td></td>
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<td>IRF7</td>
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<td>Tcells</td>
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<tr>
<td>T cell activation</td>
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<td>Tcell chemotaxis</td>
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<td>CCL5</td>
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<tr>
<td>CXCL10</td>
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<tr>
<td>Cytotoxicity</td>
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<tr>
<td>IFNG</td>
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<tr>
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<td>PRF1</td>
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<td>24</td>
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<tr>
<td>Interleukins</td>
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<td>IL1B</td>
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<td>FAS</td>
<td>0</td>
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<td>Fas Ag CD95</td>
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<td>Adhesion</td>
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<td>6</td>
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</tr>
<tr>
<td>SELP</td>
<td>0</td>
<td>6</td>
<td>24</td>
</tr>
</tbody>
</table>

Legend:
- <0.05
- 0.05-0.1
- 0.1-0.3
- 0.3-3
- 3-5
- 5-10
- 10-50
- 50-100
- 100-500
- 500-1000
- 1000-5000
- >5000
CpG as an immunoprotective agent: D35 reduces the severity of a *Leishmania amazonensis* infection

Note: Lesion size of D ODN treated animals was significantly reduced when compared to saline treated controls or macaques treated with a different ODN sequence ($p < .03$, $N=6$/group).
D35 made by GeneDesign reduces lesion size and improves healing in response to L. major infection.

https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0008050
New project funded by GHIT

Clinical development of CpG-D35 for combined treatment of cutaneous leishmaniasis

- RFP Year: 2020
- Disease: NTD (Leishmaniasis)
- Intervention: Drug
- Development Stage: Phase 1 Clinical Development
- Collaboration Partners:
  - Drugs for Neglected Diseases initiative (DNDi)
  - The University of Tokyo
  - Ajinomoto Bio-Pharma Services
  - GeneDesign (GeneDesign)
The expectation based on preclinical data is that combining CpG-D35 with chemotherapy for the treatment of patients with CL will:

1) speed lesion re-epithelization,
2) minimize scarring,
3) reduce the rate of relapse,
and hopefully
4) reduce the risk of developing drug resistance

Innovation in D35 project

Our proposed approach is clearly differentiated from the current treatment recommendations for CL.

Developing a novel D class CpG to promote the immune response required for the control of Leishmania infection, in combination with chemotherapy, will provide a major step forward over existing monotherapies or combination therapies targeting the parasite only.

Conventional chemotherapy -> KILL most Leishmania parasites

D35 promotes the HOST immune response in the host, the immune system -> LIMIT and REMOVE any remaining parasites.
Special thanks to Daniela Verthelyi at FDA and her team Dennis Klinman, Ihsan Gursel and the lab alumni

Special thanks to Byron and Steve
THANK YOU!

一期一会  ICHI GO ICHI E; Live every day as though it were last.