World Health Organization

D35 Project

FDA U.S. FOOD & DRUG ADMINISTRATION

THE INSTITUTE OF MEDICAL SCIENCE, THE UNIVERSITY OF TOKYO

Gene Design Inc.

Ajinomoto Bio-Pharma Services, GeneDesign

GHIT Fund

Global Health Innovative Technology Fund

DNDi

Drugs for Neglected Diseases *initiative*

What is CpG DNA?

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

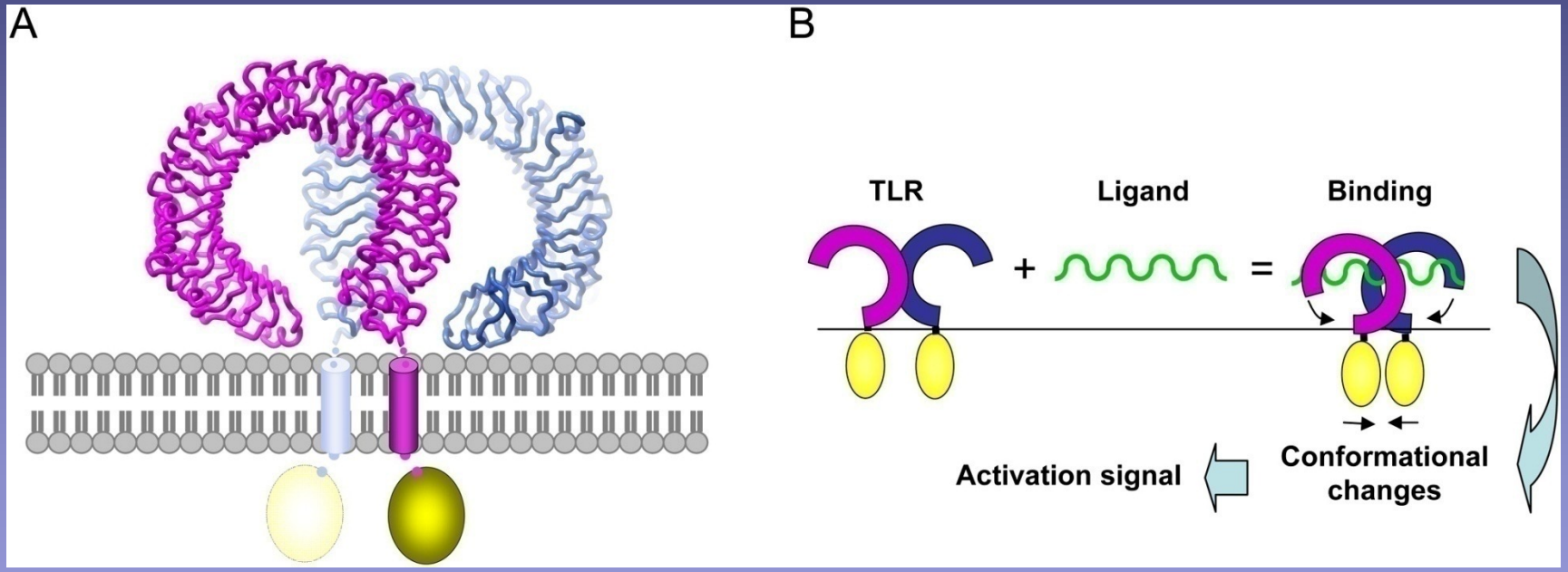
Bacterial DNA contains these CpG motifs ~20 fold more than vertebrate (mammalian) DNA due to CpG methylation and CpG suppression in mammalian DNA.

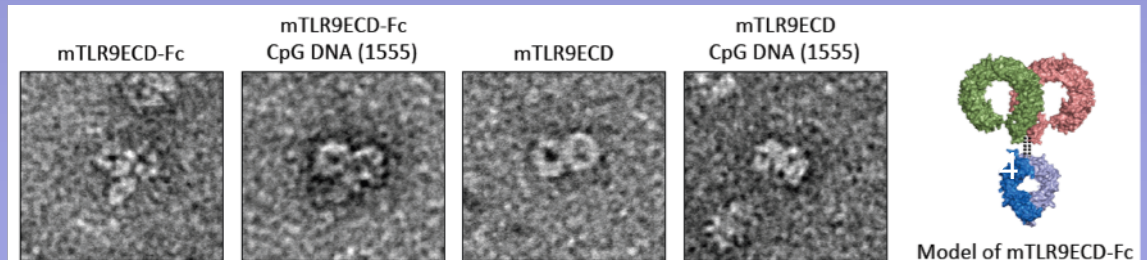
Oligonucleotides containing the CpG motifs (CpG ODN) mimic the activity of bacterial DNA.

What are CpG Motifs?

CpG motifs (mouse)	Pu-Pu-C-G-Py-Py	Immunostimulatory Activity
CpG ODN	**T-C-A-A-C-G-T-T-G-A**	**++++**
Non CpG ODN	**T-C-A-A-G-C-T-T-G-A**	**-**
CpG ODN	**T-C-G-A-C-G-T-C-G-A**	**++++**
Methyl CpG ODN	**T-C-GA-^{m}C-G-T-C-G-A**	**-**
Non CpG ODN	**T-C-T-C-C-G-G-A-G-A**	**-**

Molecular Basis of TLR-Ligand Interaction





Ishii KJ and Akira S *Cell Host Microbe* 2008

Therapeutic applications of CpG ODN

CpG ODN

↑ Innate immune activation
(↑ pDC, B, Mϕ ⇒ ↑ NK cell)

Pathogen

Tumor

↑ IFNs, IL12, NO, Chemokines
NK killing, phagocytosis

Anti-infection

Anti-Cancer

↑Th1, ↓Th2

Allergen

↓IgE, ↓Eosinophilia

Anti-Allergen

DC maturation
B cell activation

Vaccine

↑IgG2a, IFNγ, CTL

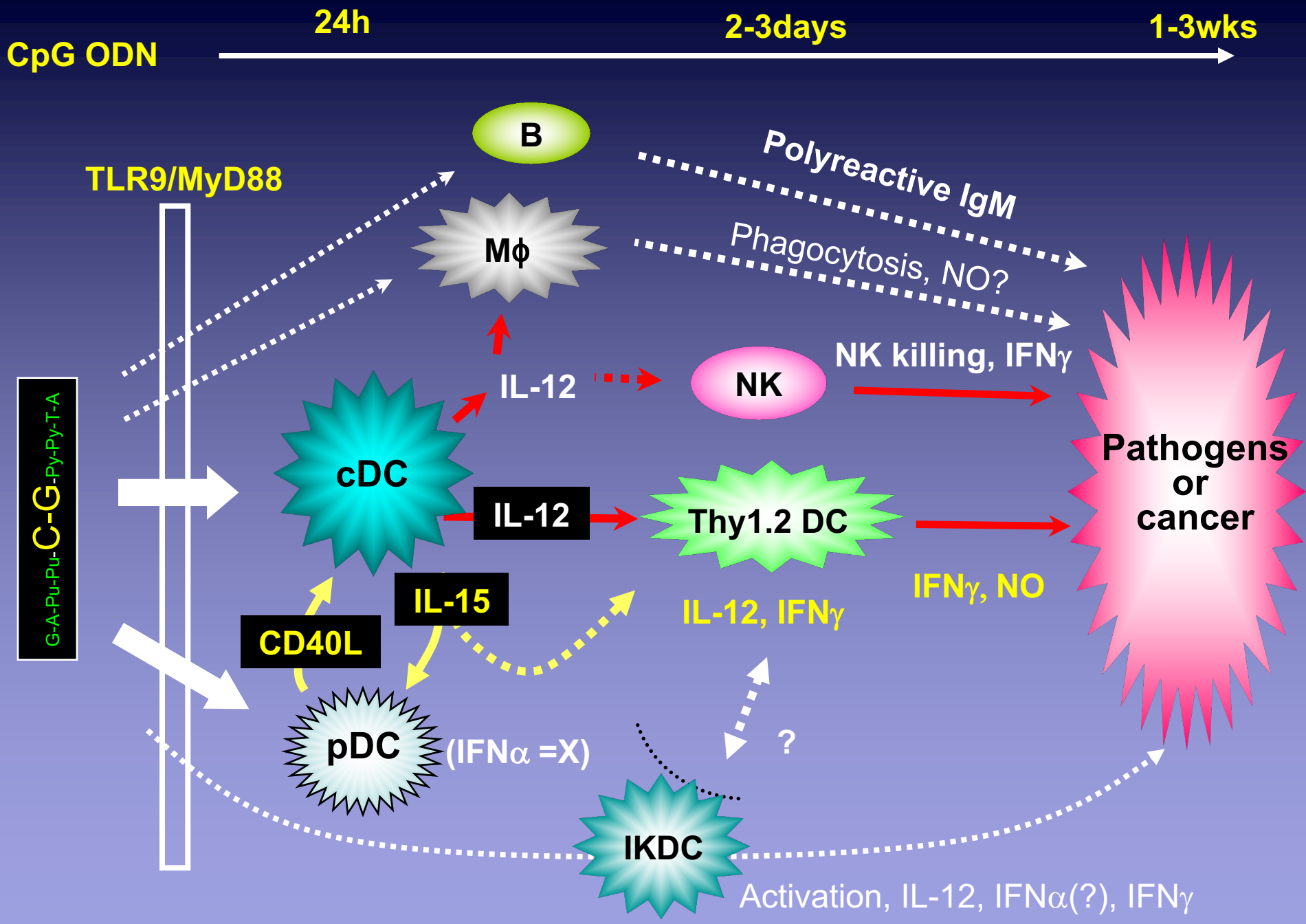
Th1 Adjuvant

Ishii KJ et al Curr. Op. Mol Ther. 2004

CpG ODN Improves Host Resistance to a Variety of Pathogens

Treatment	Organism	Challenged Dose	% protected
Saline	*L. monocytogenes*	10^3LD50	0
CpG ODN	*L. monocytogenes*	10^3	100
Saline	Malaria sporozoites	10^2	0
CpG ODN	Malaria sporozoites	10^2	88
Saline	Ebola virus	10^3	0
CpG ODN	Ebola virus	10^3	50
Saline	*P. aeruginosa*	10^2	0
CpG ODN	*P. aeruginosa*	10^2	0

Klinman DM, Verthelyi D, Takeshita F and Ishii KJ *Immunity* 1999 11(2):123-9


CpG ODN
24h
2-3days
1-3wks
B
Polyreactive IgM
TLR9/MyD88
Mϕ
Phagocytosis, NO?
NK killing, IFNγ
IL-12
NK
G-A-Pu-Pu-C-G-Py-Py-T-A
cDC
Pathogens
or
cancer
IL-12
Thy1.2 DC
IL-15
IFNγ, NO
IL-12, IFNγ
CD40L
pDC
(IFNα =X)
?
IKDC
Activation, IL-12, IFNα(?), IFNγ

CpG ODN acts as an immunoprophylaxis against variety of inf. Org.

Animal	Organism	% of protection
Mouse	*L. monocytogenes*	0 or 100
Mouse	*F. tularensis*	100
Mouse	*M. tuberculosis*	60-100
Chicken	*E. Coli*	90-100
Mouse	*P. yoelii*	80
Mouse	*L. major*	100
Rhesus macaque	*L. major*	0 (K) or 100 (D)
Mouse	Friend virus	74
Mouse	HSV-2	50
Mouse	Ebola virus	50
Mouse	RML prion	100

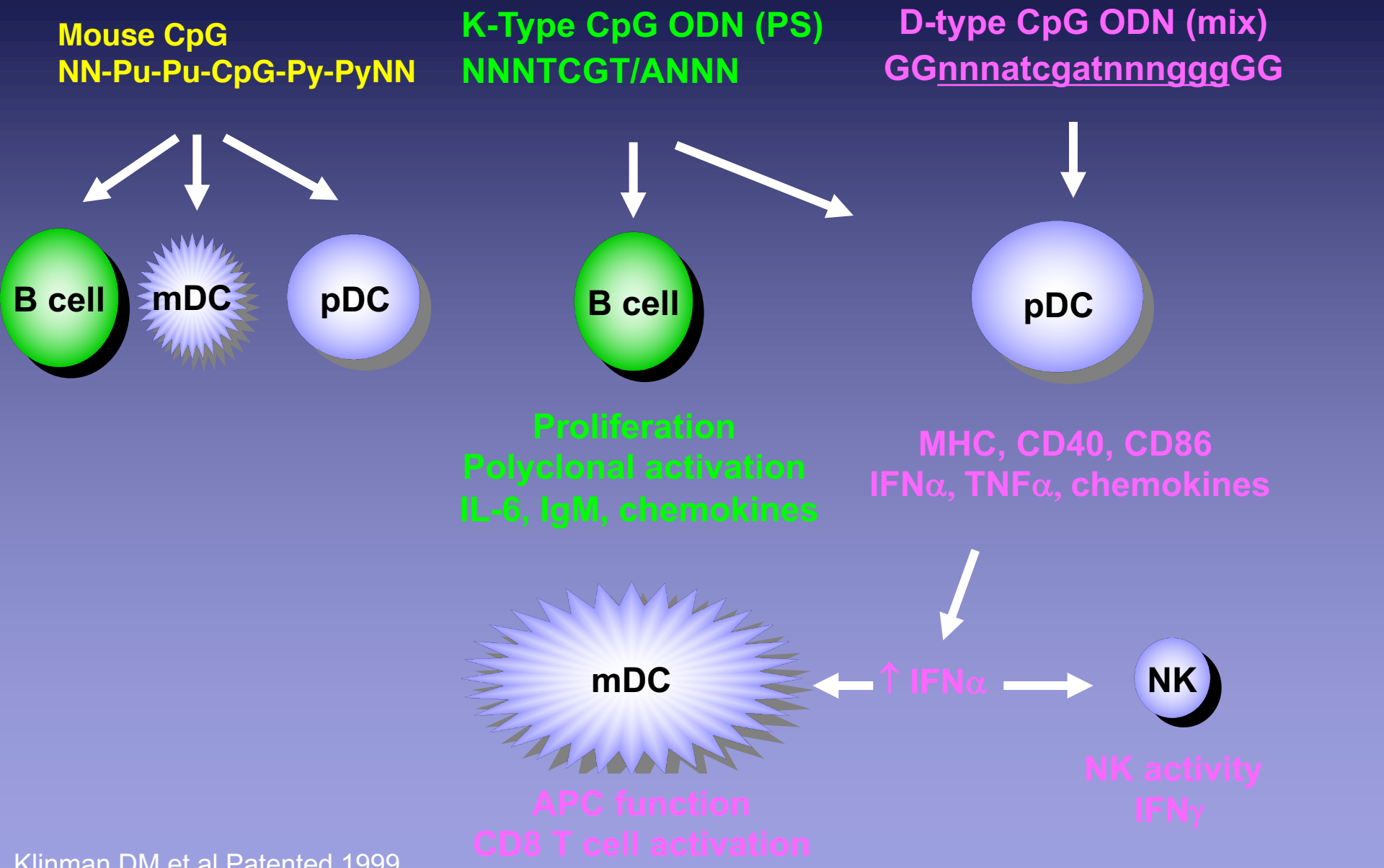
Ishii KJ et al., Curr Opin Mol Ther. 2004 6(2):166-74.

Obstacles

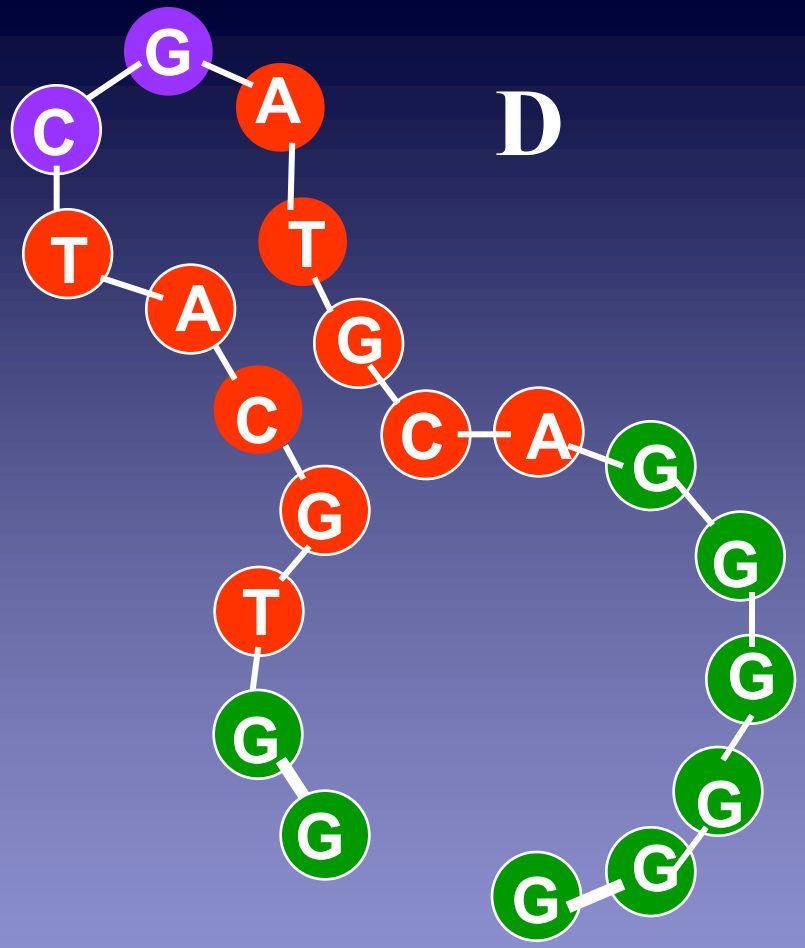
- Optimal CpG motifs for mouse are not active in human immune system.
- Low efficacy of DNA vaccines are observed currently in human clinical trials (than that of mouse).
- TLR9 expression in immune cells is different between human and mouse .

→Needs humanized CpG motifs

Humanization of CpG DNA (ODN): Distinct types of CpG



Klinman DM et al Patented 1999,
Verthelyi D, Ishii KJ et al J. Immunol. 2001, Ishii KJ et al *Curr Opin Mol Ther*. 2004 6(2):166-74.



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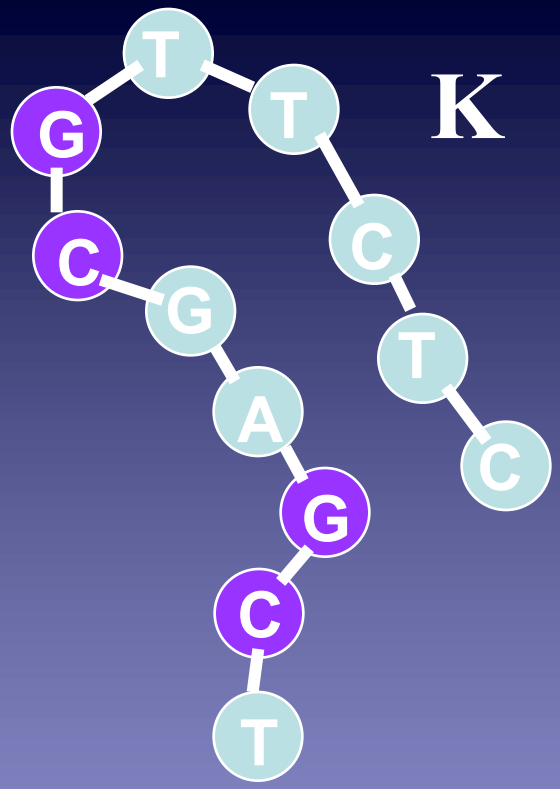

K

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.

	MPL (TLR4)	K ODN (TLR9)	D35 ODN (TLR9)	
Proliferation	+++	+++	-	(B cells)
IgM	+++	+++	-	(B cells)
IL-10	++	++	+	(B cells)
TNFα	+++	+++	+/-	(Monocytes)
IL-6	+++	+++	++	(Monocytes)
IP-10	+	++	+++	(Monocytes)
IFNα	+	-	++++	(pDC)
IFNγ	+	+	++++	(NK cells)

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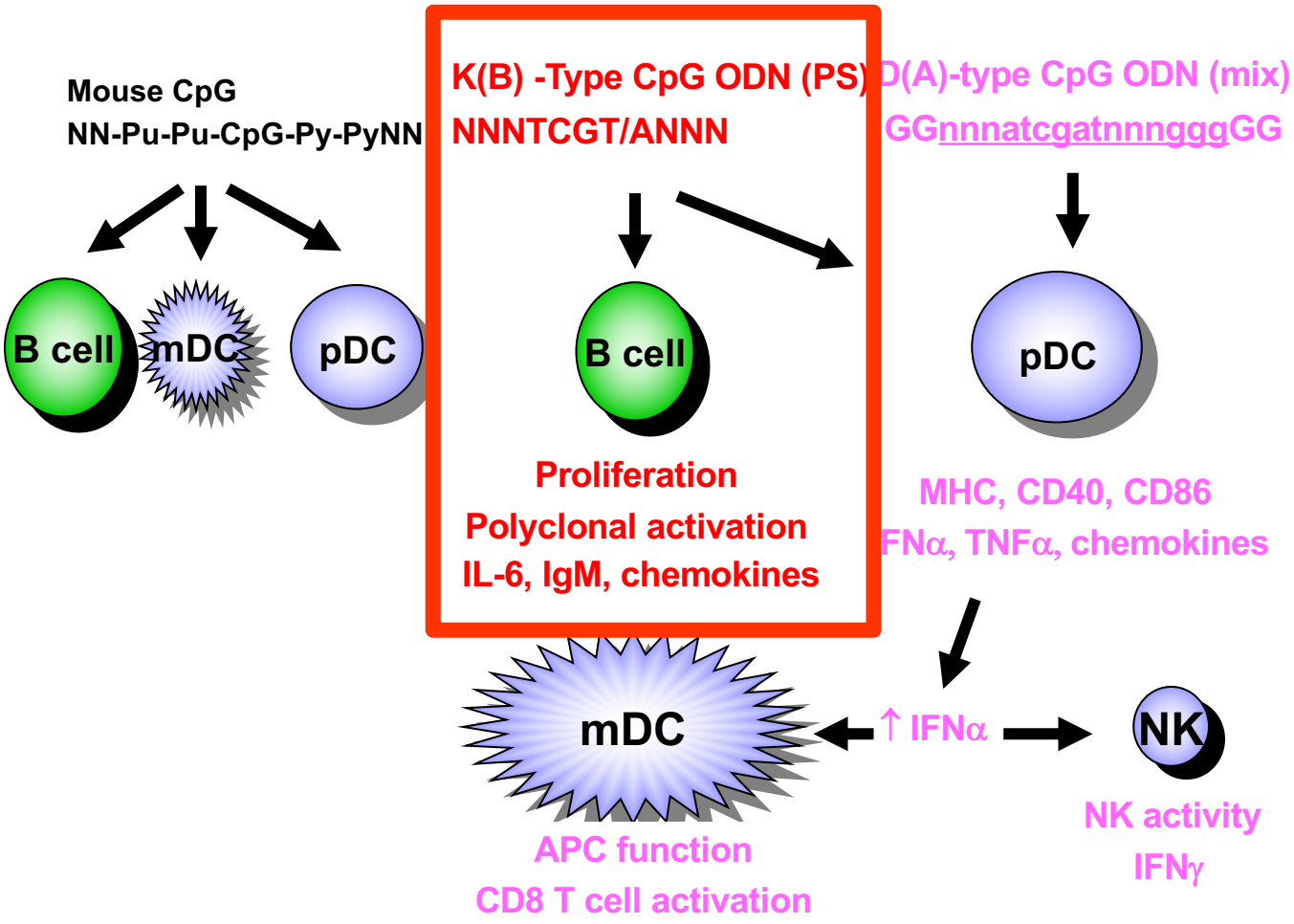
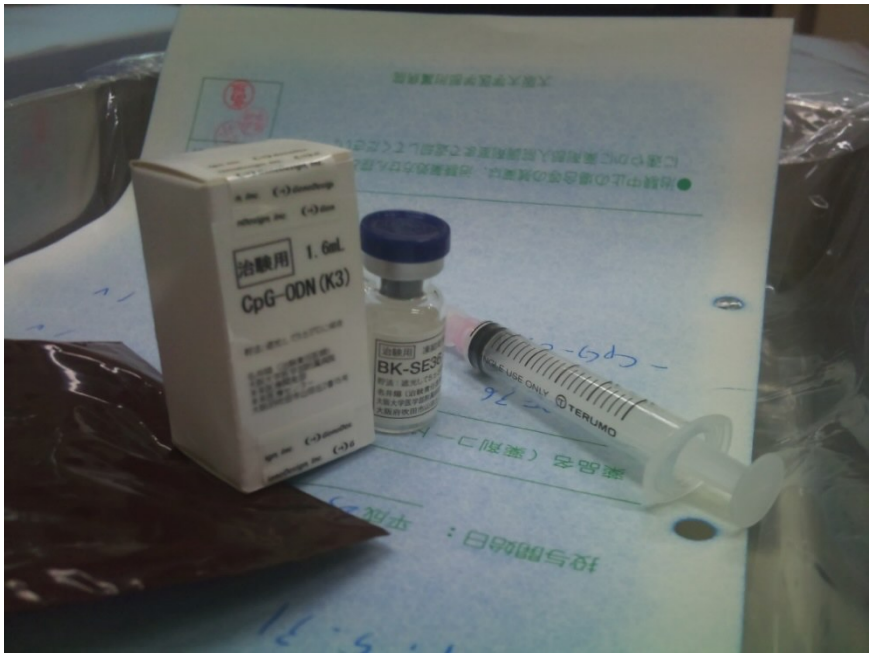


Ishii KJ, Gursel I, Gursel M, Klinman DM. ***Curr Opin Mol Ther***. 2004 6(2):166-74. Ishii KJ and Akira S ***Trends in Immunology*** 2006

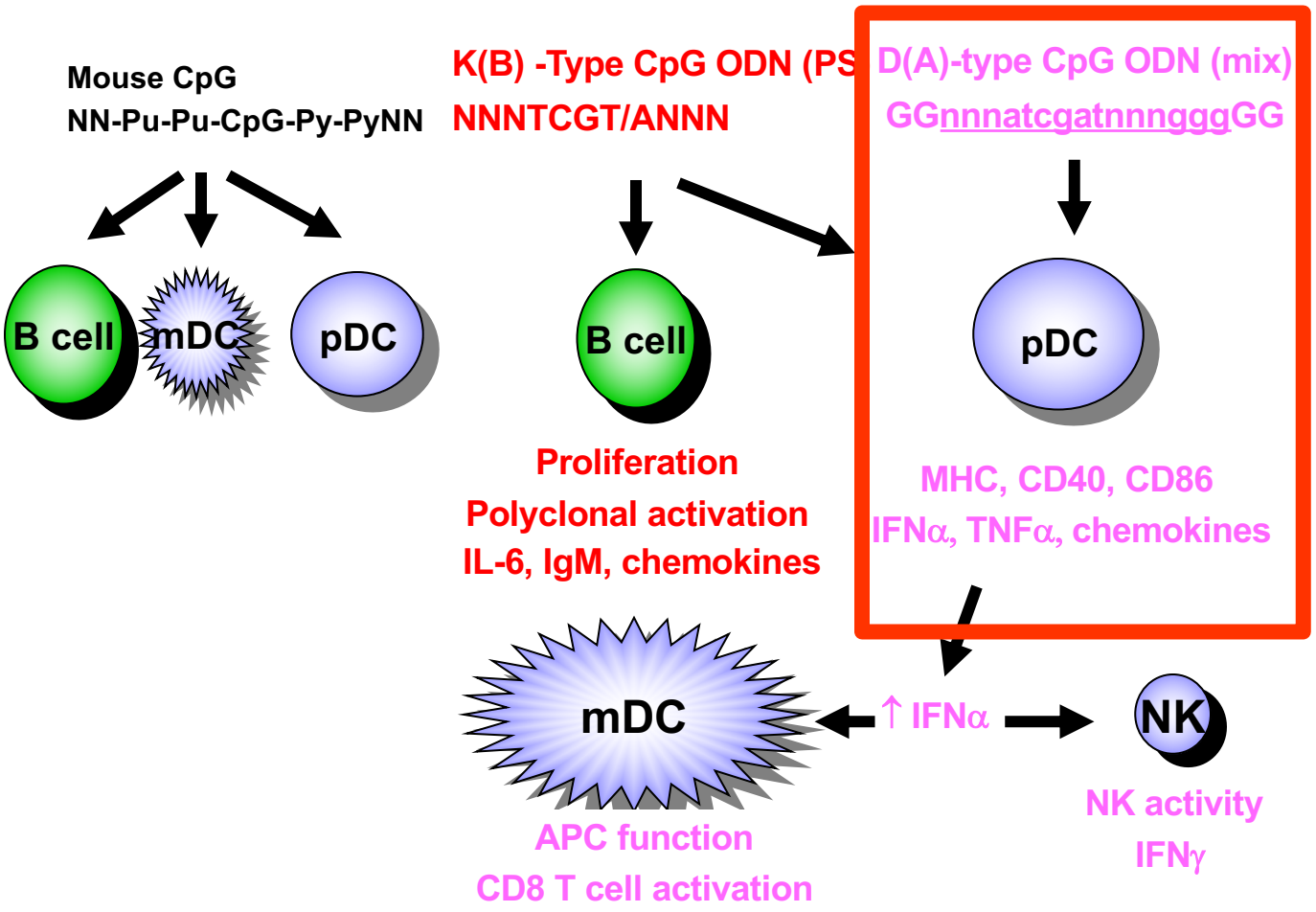
K3 is on the way to human

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

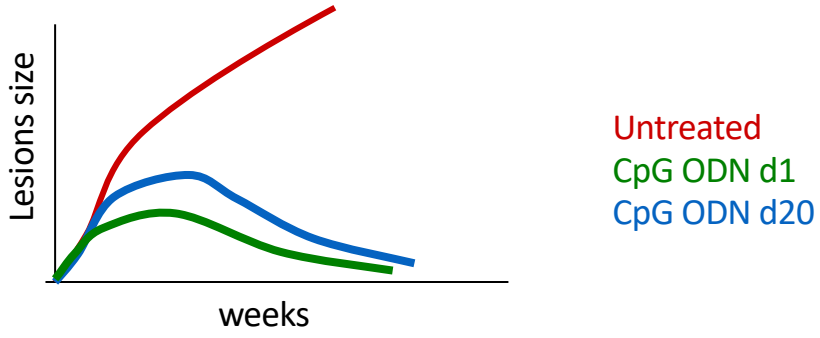


Ishii KJ, Gursel I, Gursel M, Klinman DM. ***Curr Opin Mol Ther***. 2004 6(2):166-74. Ishii KJ and Akira S ***Trends in Immunology*** 2006

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

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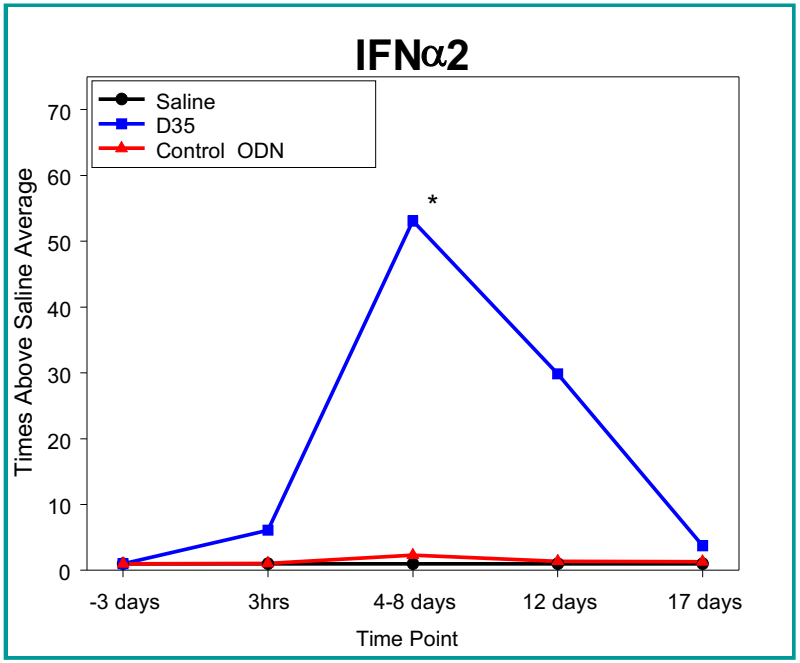
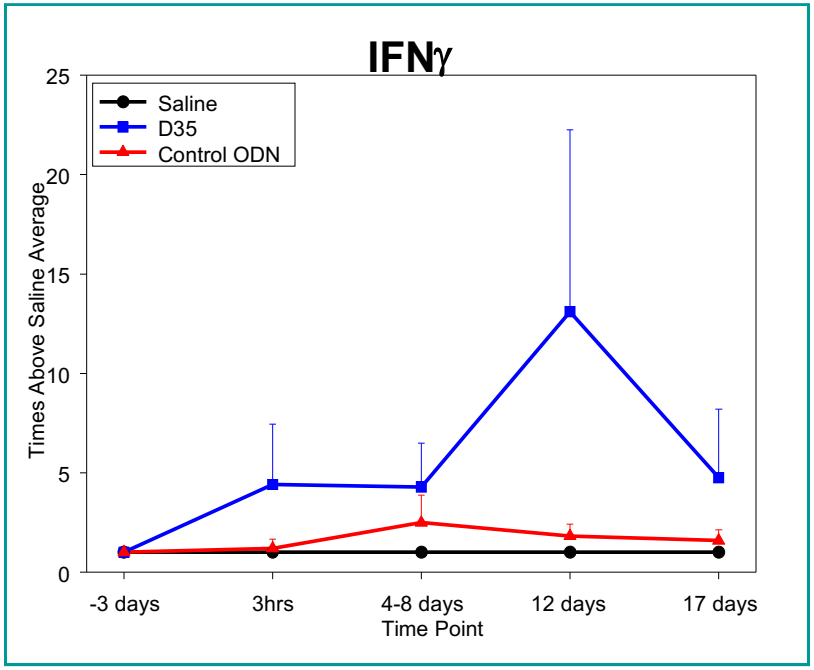
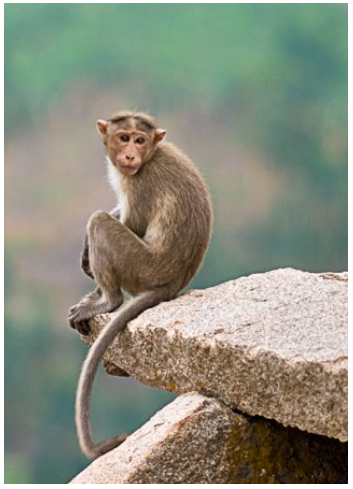
How about Monkey?

In vivo D35 induces increase levels of IFNα and IFNγ in serum

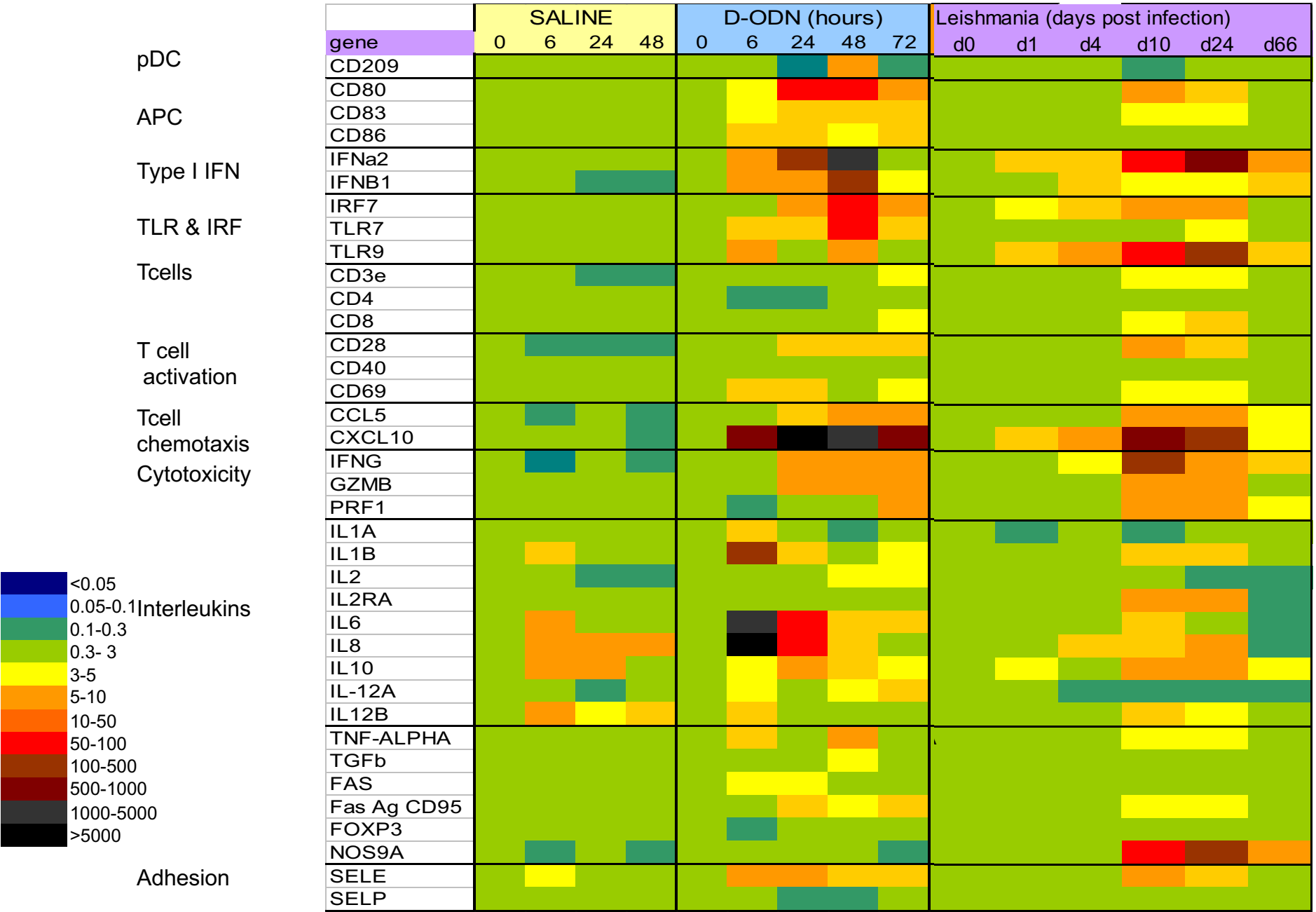




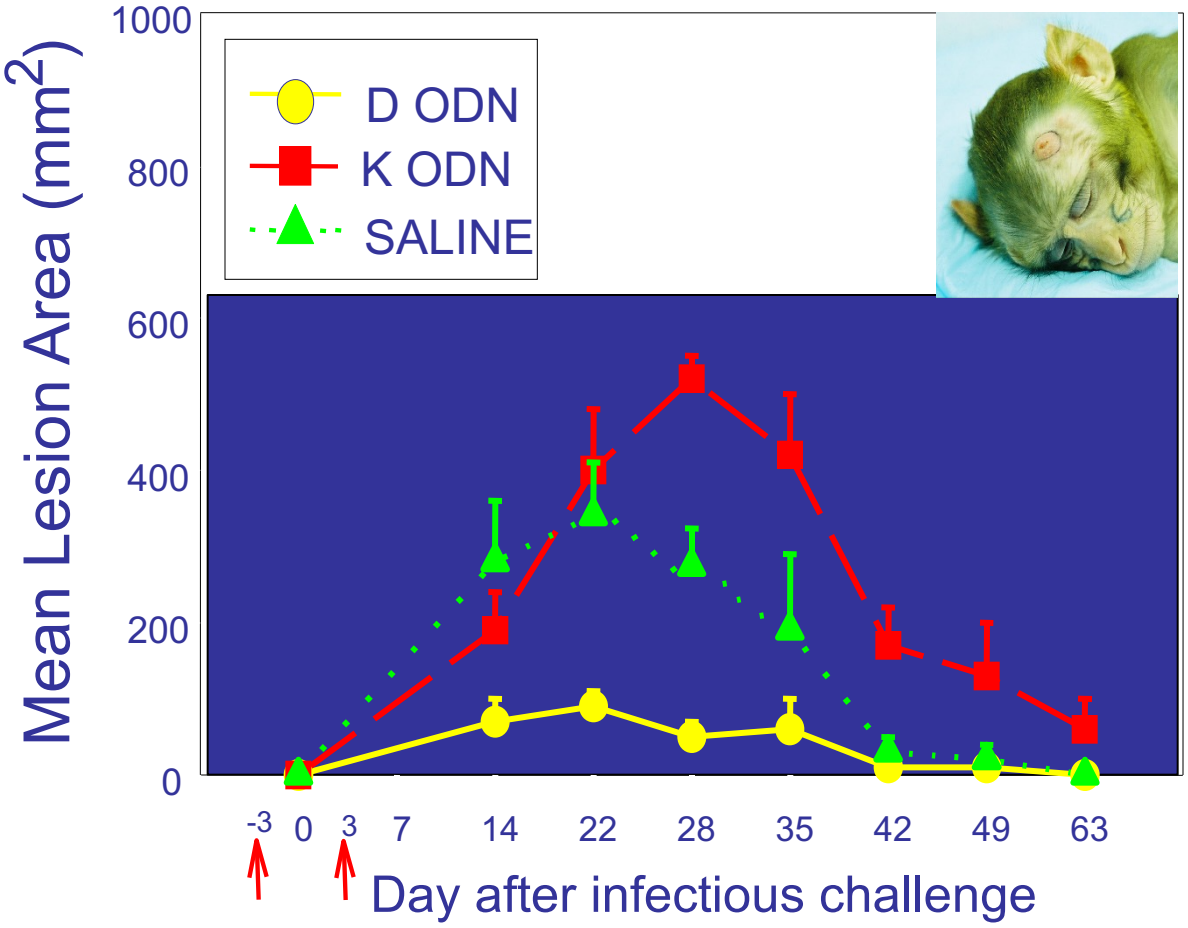
Macaque treated *in vivo* with D35
(SC 0.5mg/kg on days 1, 3 & 7).
5 macaques per group.



Fold increase in gene expression in skin over time in macaques injected with D35 or L. major (note response in hours vs. days)



CpG as an immunoprotective agent: D35 reduces the severity of a Leishmania amazonensis infection

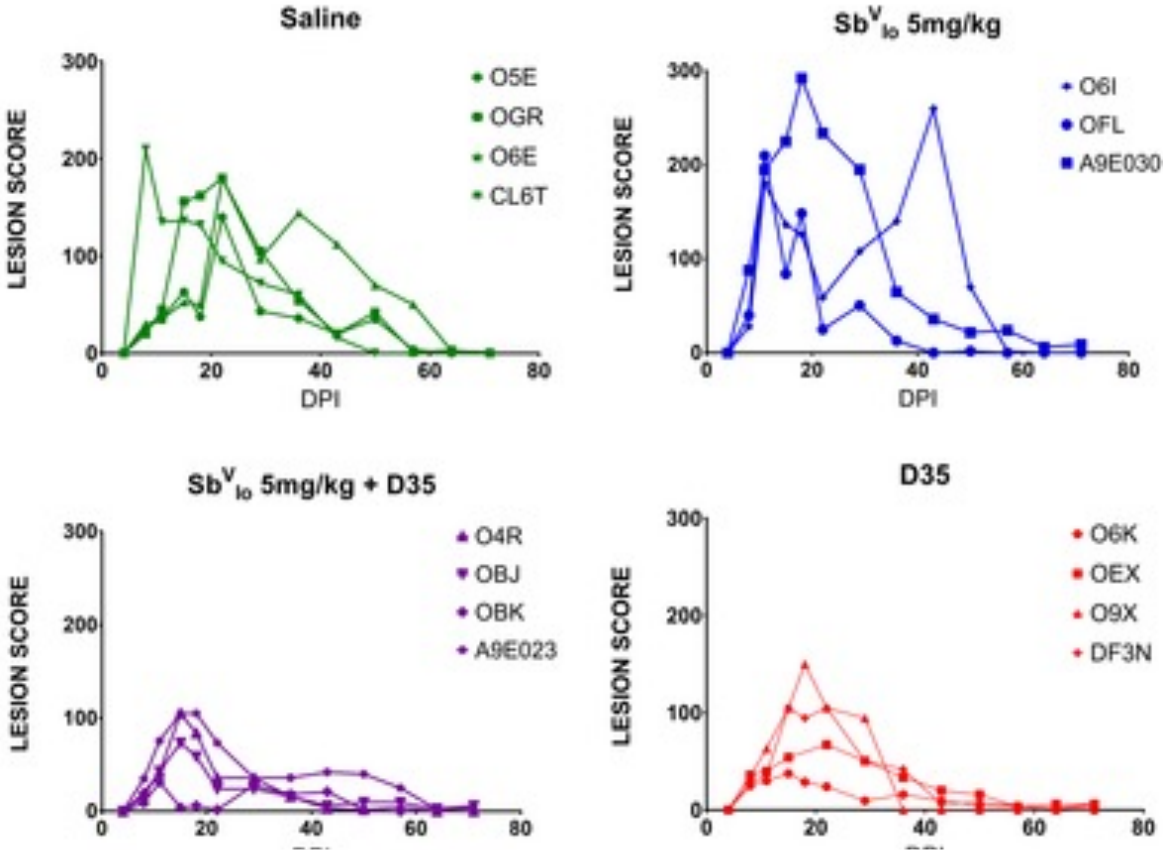


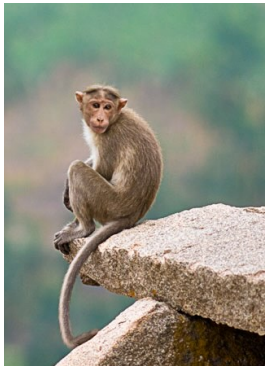
Note: Lesion size of D ODN treated animals was significantly reduced when compared to saline treated controls or macaques treated with a different ODN sequence ($p < .03$, N=6/group).

J.Immunol.2003

D35 made by GeneDesign
reduces lesion size and improves healing in response to L. major infection.

A





Thacker SG, McWilliams IL, Bonnet B, Halie L, Beaucage S, et al. (2020) CpG ODN D35 improves the response to abbreviated low-dose pentavalent antimonial treatment in non-human primate model of cutaneous leishmaniasis. PLOS Neglected Tropical Diseases 14(2): e0008050. https://doi.org/10.1371/journal.pntd.0008050
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)

D35 project will address global health challenges

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

1. Flynn B, Wang V, Sacks DL, Seder RA, Verthelyi D. 2005. Prevention and treatment of cutaneous leishmaniasis in primates by using synthetic type D/A oligodeoxynucleotides expressing CpG motifs. Infect Immun;73(8):4948-54.

2. Verthelyi D, Gursel M, Kenney RT, Lifson JD, Liu S, Mican J, Klinman DM. 2003. CpG oligodeoxynucleotides protect normal and SIV-infected macaques from Leishmania infection. J Immunol; 170(9):4717-23.

3. Miranda-Verastegui C, Tulliano G, Gyorkos TW, Calderon W, Rahme E, Ward B, Cruz M, Llanos-Cuentas A, Matlashewski G. 2009. First-line therapy for human cutaneous leishmaniasis in Peru using the TLR7 agonist imiquimod in combination with pentavalent antimony. PLoS Negl Trop Dis; 3(7):e491. doi: 10.1371/journal.pntd.0000491

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.