Vaccine Forum 2010

アジュバント・ワークショップ

「α-GalCerアジュバントの免疫制御メカニズムと臨床応用」

石井　保之
独立行政法人理化学研究所
免疫・アレルギー科学総合研究センター（RCAI)
ワクチンデザイン研究チーム

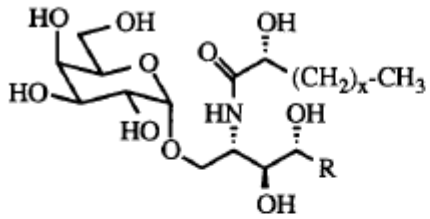
α-GalCer（α-galactosylceramide）の発見

- 旧キリンビール医薬探索研究所のチームが、胆癌マウスを用いたin vivoアッセイで、抗腫瘍効果を示す天然物化合物アゲラスフィン類（α–GalCer）を沖縄海域に生息する海綿から単離同定した。
- その後、様々な誘導体を合成し、最も抗腫瘍効果が期待できるα-GalCer (KRN7000)を選択した。

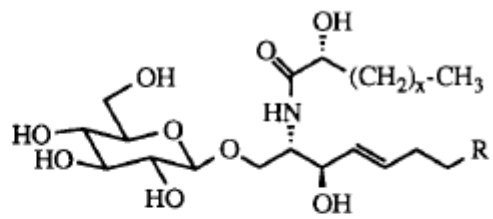
- 1993: Natori T, Koezuka Y, Higa T. Agelasphins, “Novel α-galactosylceramides from the marine sponge agelas mauritianus”. *Tetrahedron letters*, 34:5591.
- 1994: Natori T, Morita M, Akimoto K, Koezuka Y. “Agelasphins, novel antitumor and immunostimulatory cerebrosides from the sponge *Agelas mauritanus*.” *Tetrahedron*, 50:2771.
- 1995: Morita M, Natori T, Akimoto K, Osawa T, Fukushima H, Koezuka Y. “Syntheses of a-, b-monoglycosylceramide and four diastereomers of an α-galactosylceramide.“ *Bioorganic & Medical Chemistry Letters*, 5:699.
- 1995: Kobayashi E, Motoki K, Uchida T, Fukushima H, Koezuka Y. “KRN7000, a novel immunomodulator, and its antitumor activities.” *Oncol Res*, 7:529.
- 1995: Morita M, Motoki K, Akimoto K, et al. ”Structure-activity relationship of α-galactosylceramides against B16-bearing mice.” *J Med Chem* , 38:2176.

α-GalCerの構造と活性

天然物



	X	R
AGL-7a	21	$-(CH_2)_{11}-CH_3$
AGL-9b	21	$-(CH_2)_{11}-CH(CH_3)_2$
AGL-11	21	$-(CH_2)_{11}-CH(CH_3)-C_2H_5$
AGL-13	22	$-(CH_2)_{11}-CH(CH_3)-C_2H_5$



	X	R
AGL-10	21	$-(CH_2)_6-CH_3$ (CH$_3$ branch)

Fig. 1. Structures of AGL-7a, AGL-9b, AGL-11, AGL-13, and AGL-10.

Natori et al. 1994 *Tetrahedron*

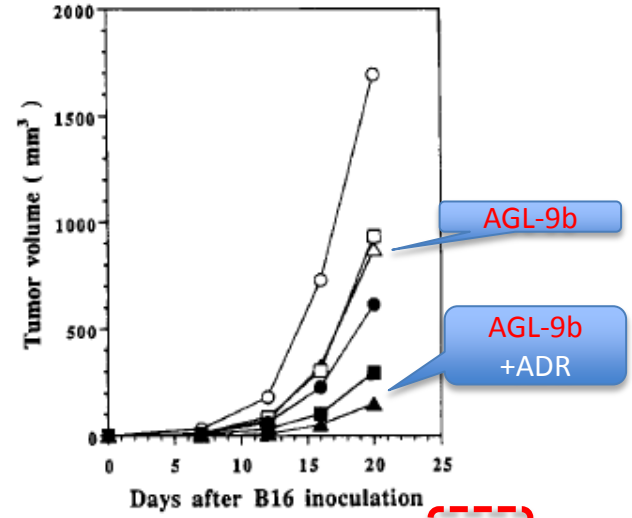


Figure 1. Tumor growth inhibitory effects of AGL-9b and AGL-502 in combination with or without adriamycin on mice subcutaneously inoculated with B16 cells. B16 cells (1×10^6) were subcutaneously inculated into female BDF_1 mice on day 0. AGL-9b and AGL-502 were intravenously administered on days 1, 5, and 9, and ADR was intraperitoneally administered on day 1. The mean of six mice is shown here: ○, control; △, AGL-9b (100 μg/kg); □, AGL-502 (100 μg/kg); ●, ADR (10 mg/kg); ▲, ADR + AGL-9b; ■, ADR + AGL-502.

Morita et al. 1995 *J Med Chem*

化学合成物

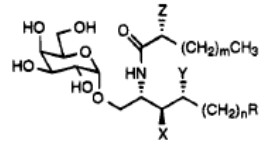
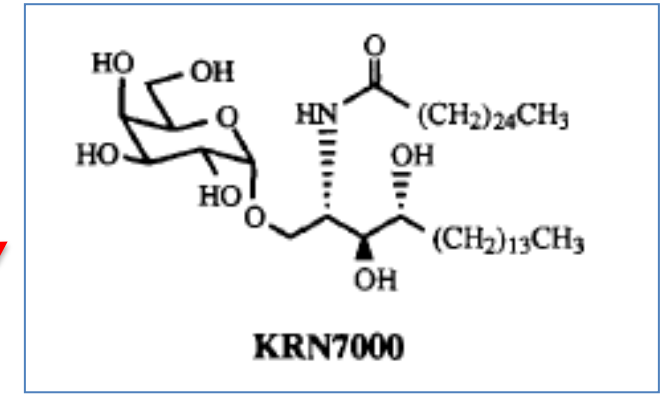


Table 3. Tumor Growth Inhibitory Effects of AGL Analogues on Mice Subcutaneously Inoculated with B16 Cells

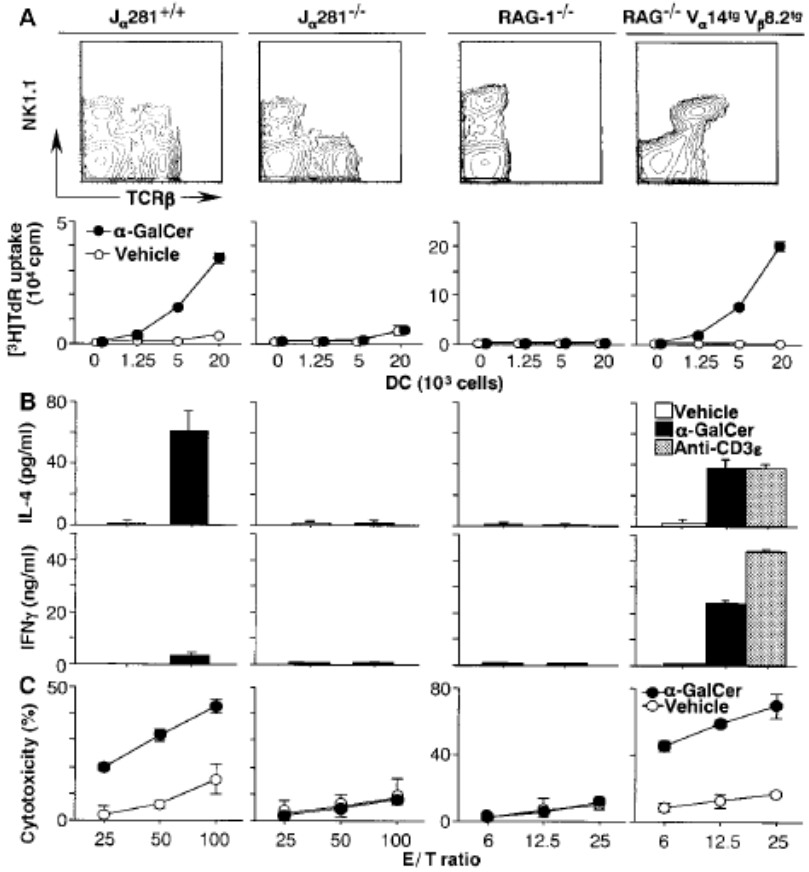
							maximum TGIR (%)		
compd	X	Y	Z	R	*m*	*n*	single	MMC	AGLs + MMC
AGL-502	OH	OH	OH	$CH(CH_3)_2$	21	11	86.8	61.5	94.6
AGL-519	OH	OH	OH	CH_3	21	12	82.4	61.5	90.6
AGL-509	OH	OH	OH	CH_3	21	6	46.2	46.5	85.1
AGL-510	OH	OH	OH	CH_3	21	10	94.1	46.5	77.4
AGL-512	OH	OH	OH	CH_3	21	13	57.9	64.0	91.9
AGL-548	OH	OH	OH	CH_3	23	13	92.8	87.3	97.7
AGL-549	OH	OH	OH	CH_3	23	14	72.3	87.3	97.1
AGL-550	OH	OH	OH	CH_3	23	15	92.8	87.3	94.3
AGL-512	OH	OH	OH	CH_3	21	13	57.9	64.0	91.9
AGL-525	OH	OH	H	CH_3	21	13	65.0	64.8	90.8
AGL-506	OH	H	OH	CH_3	21	13	81.3	70.7	98.5
AGL-514	OH	H	H	CH_3	21	13	68.9	64.0	99.1
AGL-535[a]	H	H	H	CH_3	21	13	40.7	80.0	91.4
AGL-517	OH	H	H	CH_3	11	13	54.2	64.1	84.4
AGL-536	OH	H	H	CH_3	15	13	78.1	64.1	80.3
AGL-544	OH	H	H	CH_3	17	13	53.1	39.4	90.4
AGL-543	OH	H	H	CH_3	19	13	56.9	39.4	85.0
AGL-514	OH	H	H	CH_3	21	13	68.9	64.0	99.1
AGL-548	OH	OH	OH	CH_3	23	13	69.8	67.6	85.0[b]
AGL-582	OH	OH	H	CH_3	23	13	53.8	67.6	86.7[b]

[a] C2 epimeric mixture. [b] AGL-548 and AGL582 were administered at days 6, 10, and 14. Tumor volume of each mouse was measured on days 8, 12, 16, and 20, and maximum TGIRs are shown in this table.

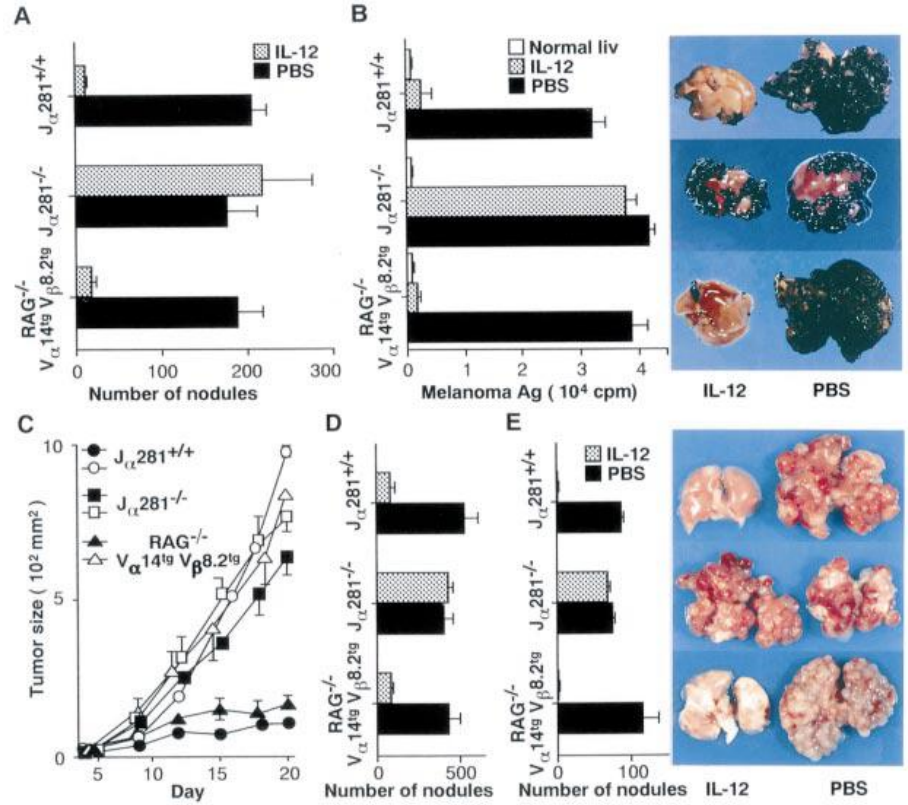


α-GalCerはVα14 iNKT細胞のリガンドであった

- 1997: Kawano T, Cui J, Koezuka Y, et al. “CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides.” *Science* , 278:1626.



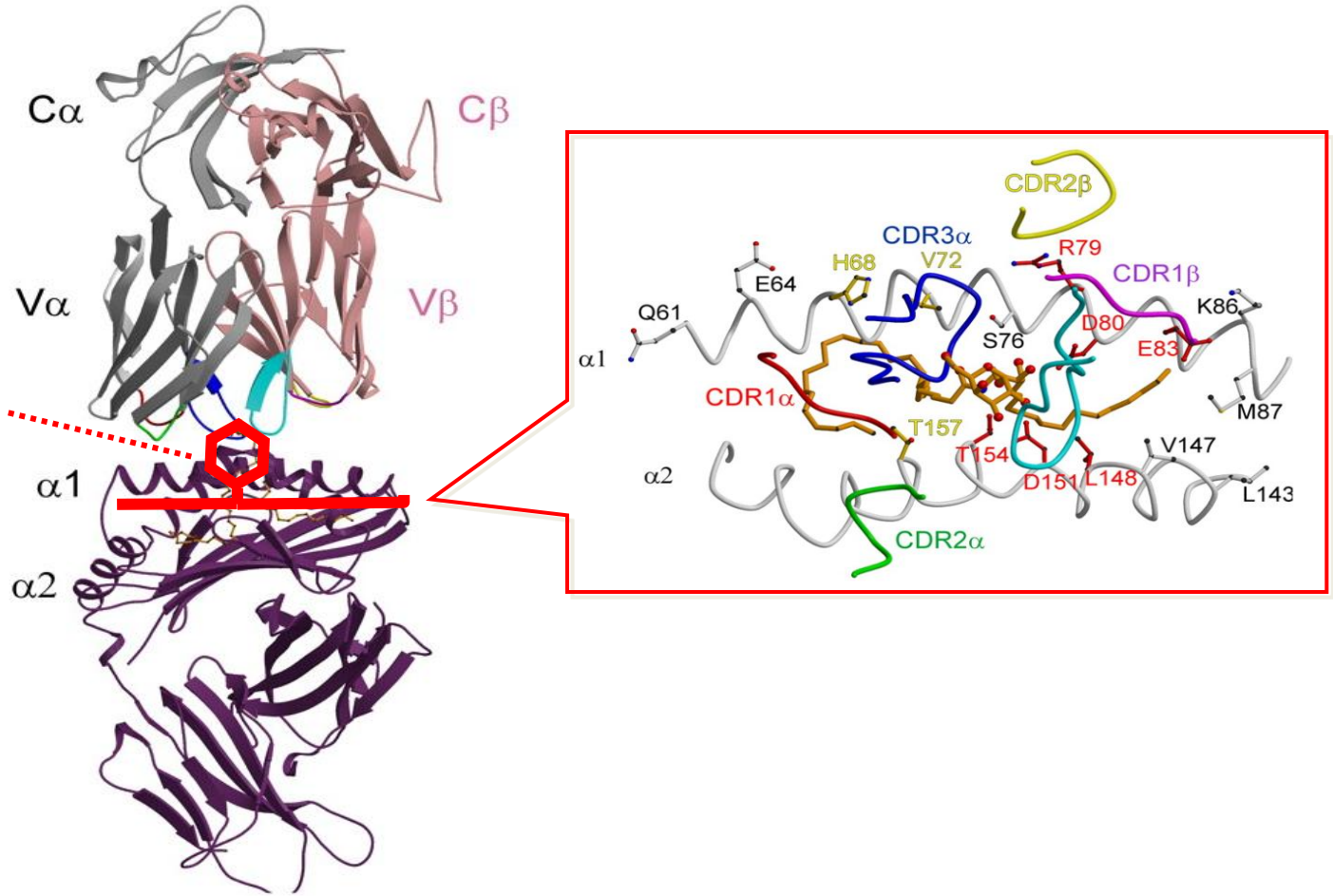
- 1997: Cui J, Shin T, Kawano T, Sato H, et al. “Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors.” *Science* , 278:1623.



α-GalCerはCD1d分子提示され、invariant TCRα 鎖を発現するiNKT細胞を刺激する

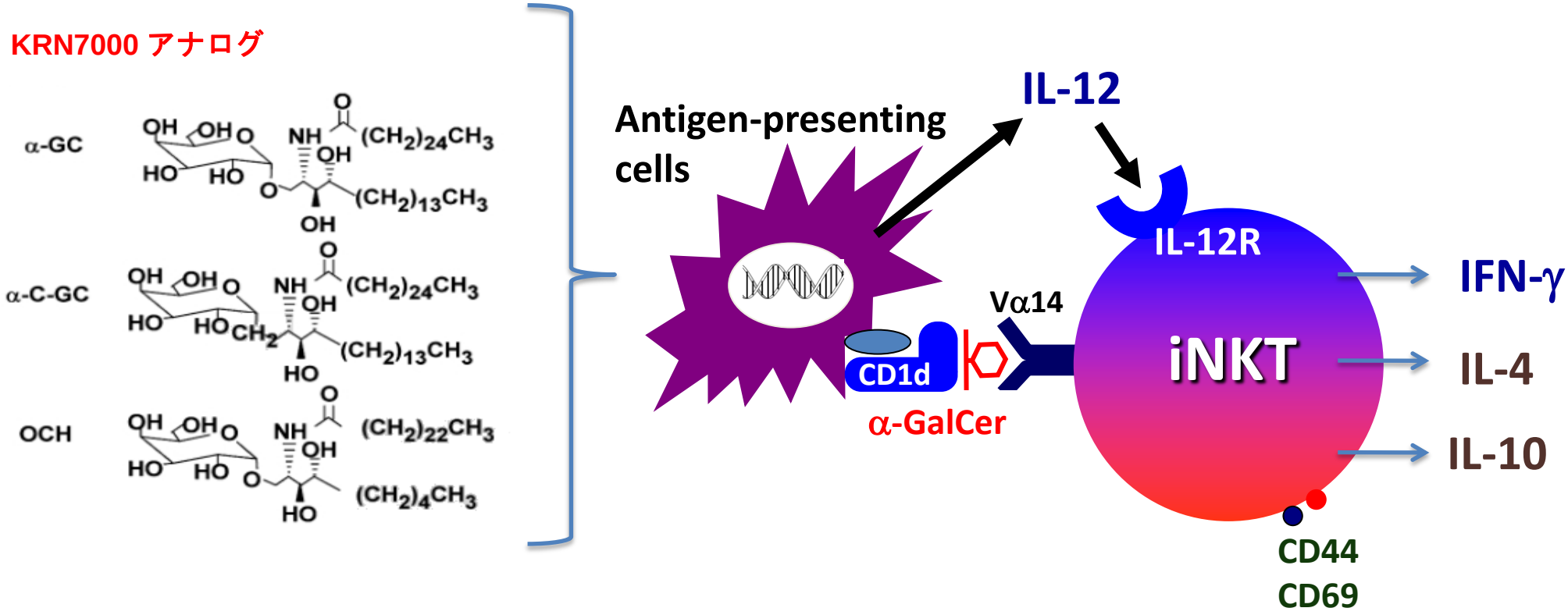
Vα14 (Mouse)
Vα24 (Human)

α-GalCer (KRN7000)



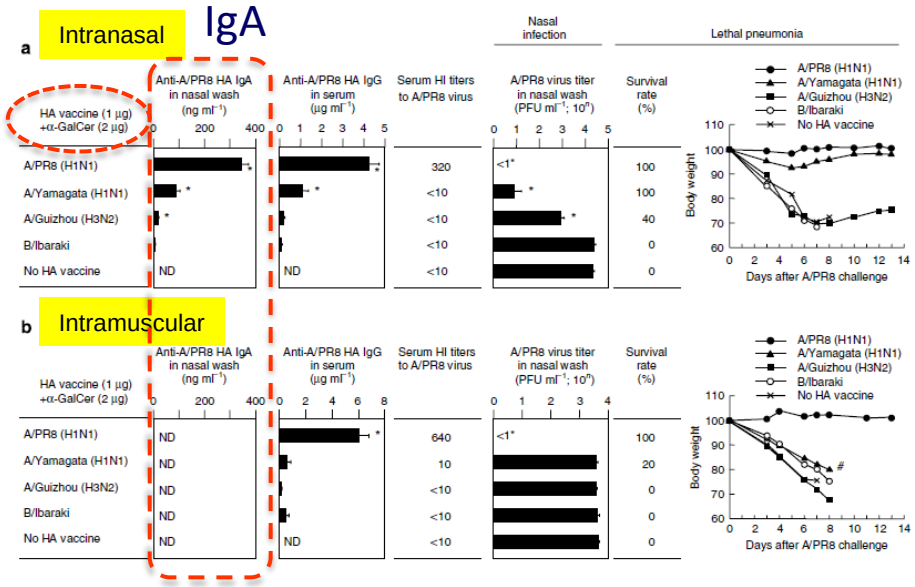
JEM, Volume 203, Number 3, 661

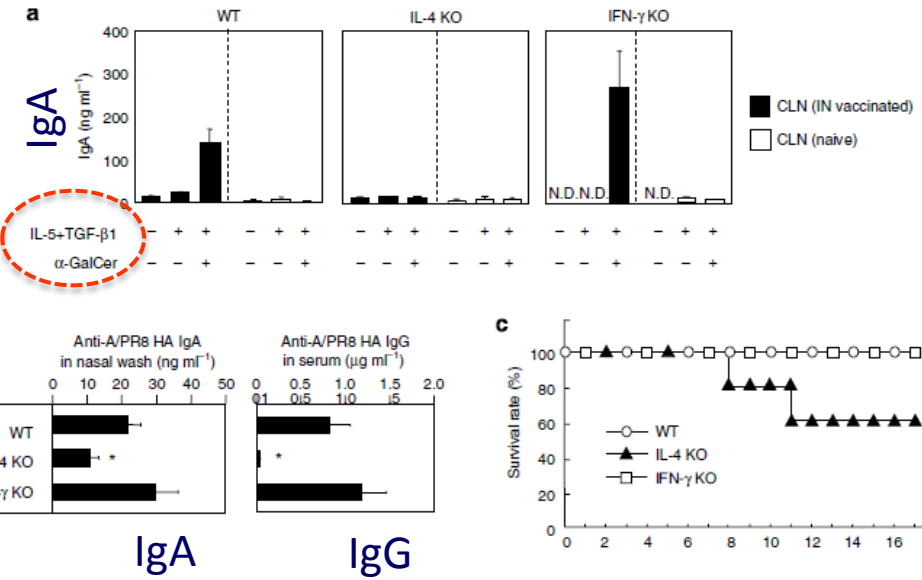
α-GalCerによるVα14 iNKT細胞の活性化



感染症に対する有効性

- マウスにα-GalCerと抗原を経鼻投与するとIgA産生が誘導される。
  - NKT細胞からのIL-4産生が重要

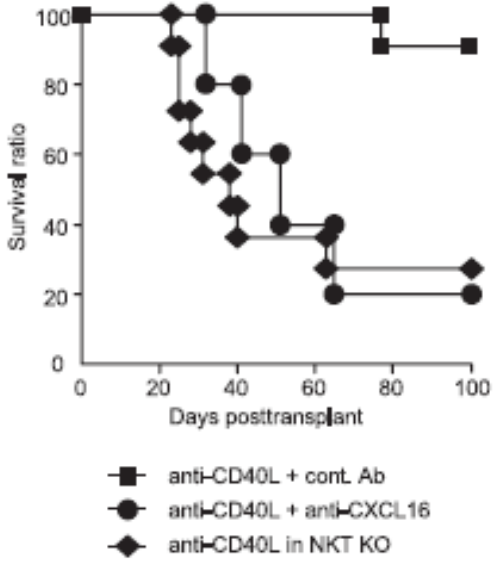




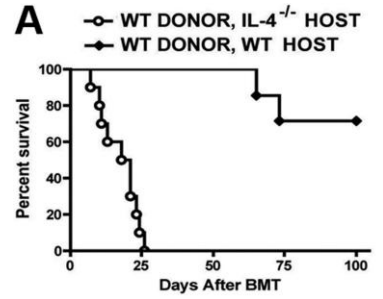
- 2005: Ko S-Y, Ko H-J, Chang W-S et al. “α-galactosylceramide can act as a nasal vaccine adjuvant inducing protective immune responses against viral infection and tumor. *“ J Immunol*, 175:3309.
- 2008: Kamijuke H, Nagata T, Jiang X et al. “Mechanism of NKT cell activation by intranasal coadministration of α-galactosylceramide, which can induce cross-protection against influenza viruses.” *Muc Immunity*, 1:208.
- 2010: Noda K, Kodama S, Umemoto S et al, “Nasal vaccination with P6 outer membrane protein and α-galactosylceramide induces nontypeable *Haemophilus influenzae*-specific protective immunity associated with NKT cells activation and dendritic cell expansion in nasophaynz.” *Vaccine*, 28:5068.

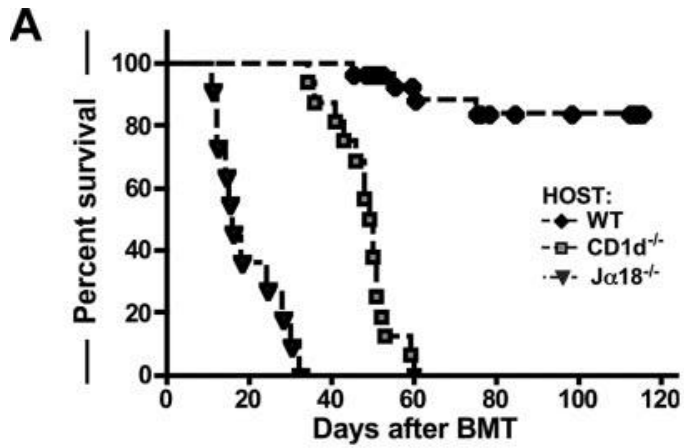
移植拒絶やGvHDをNKT細胞が保護

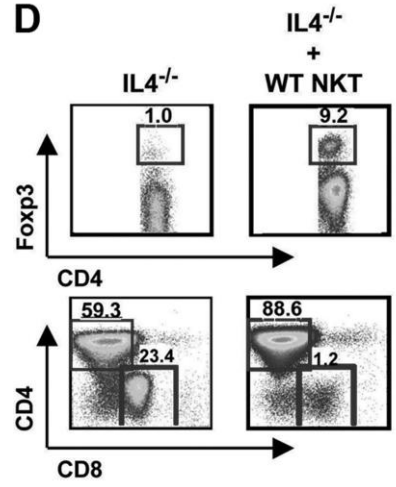
- NKT欠損マウスへの心臓移植モデルでは抗CD40L抗体による移植拒絶抑制が認められない。
  - NKT細胞が必須



- マウス骨髄移植モデルでは、レシピエントのNKT細胞がGvHD抑制に働く
  - NKT細胞のIL-4産生
  - Foxp3+CD4+Tregの増殖



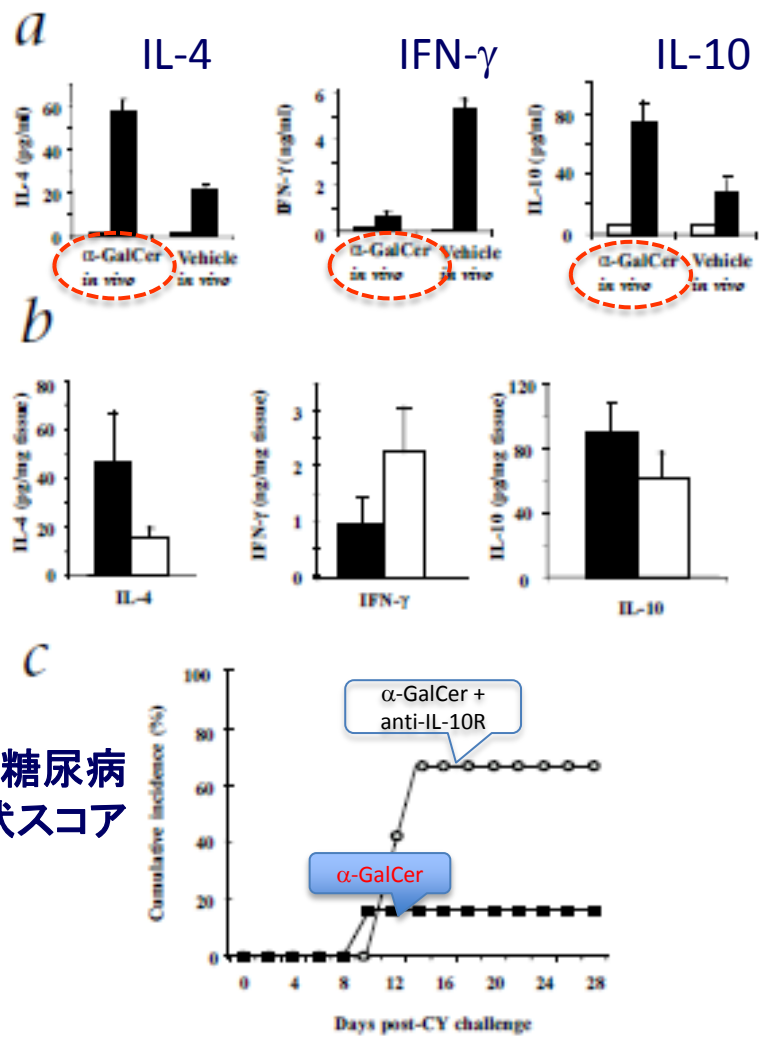


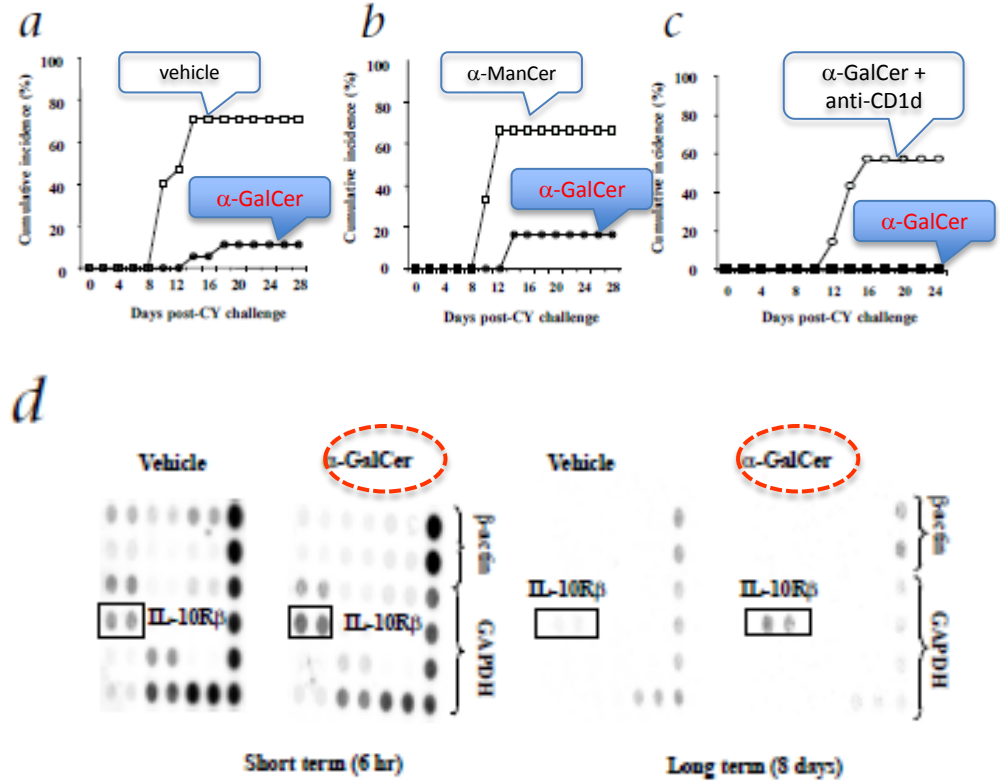


- 2000: Seino K-I, Fukao K, Muramoto K, et al. "requirement for natural killer T (NKT) cells in the induction of allograft tolerance." *Proc Natl Acad Sci USA,* 98:2577.
- 2005: Jiang X, Shimaoka T, Kojo S, et al. "Critical role of CXCL16/CXCR6 in NKT cell trafficking in allograft tolerance." *J Immunol*, 175:2051.
- 2007: Pillai AB, George TI, Dutt S, Strober S, "Host NKT cells can prevent graft-versus-host disease and permit graft antitumor activity after bone marrow transplantation." *J Immunol,* 178:6242.
- 2009: Pillai AB, George TI, Dutt S, Strober S, "Host natural killer T cells induce an interleukin-4-dependent expansion of おdonot CD4+CD25+Foxp3+ T regulatory cells that protects against graft-versus-host disease." *Blood* 113:4458.

I型糖尿病に対する有効性

- NODマウスにα-GalCerをi.p.連投すると、糖尿病症状が抑えられる。
  - NKT細胞のIL-4とIL-10産生が増強
  - IL-10R発現が増強

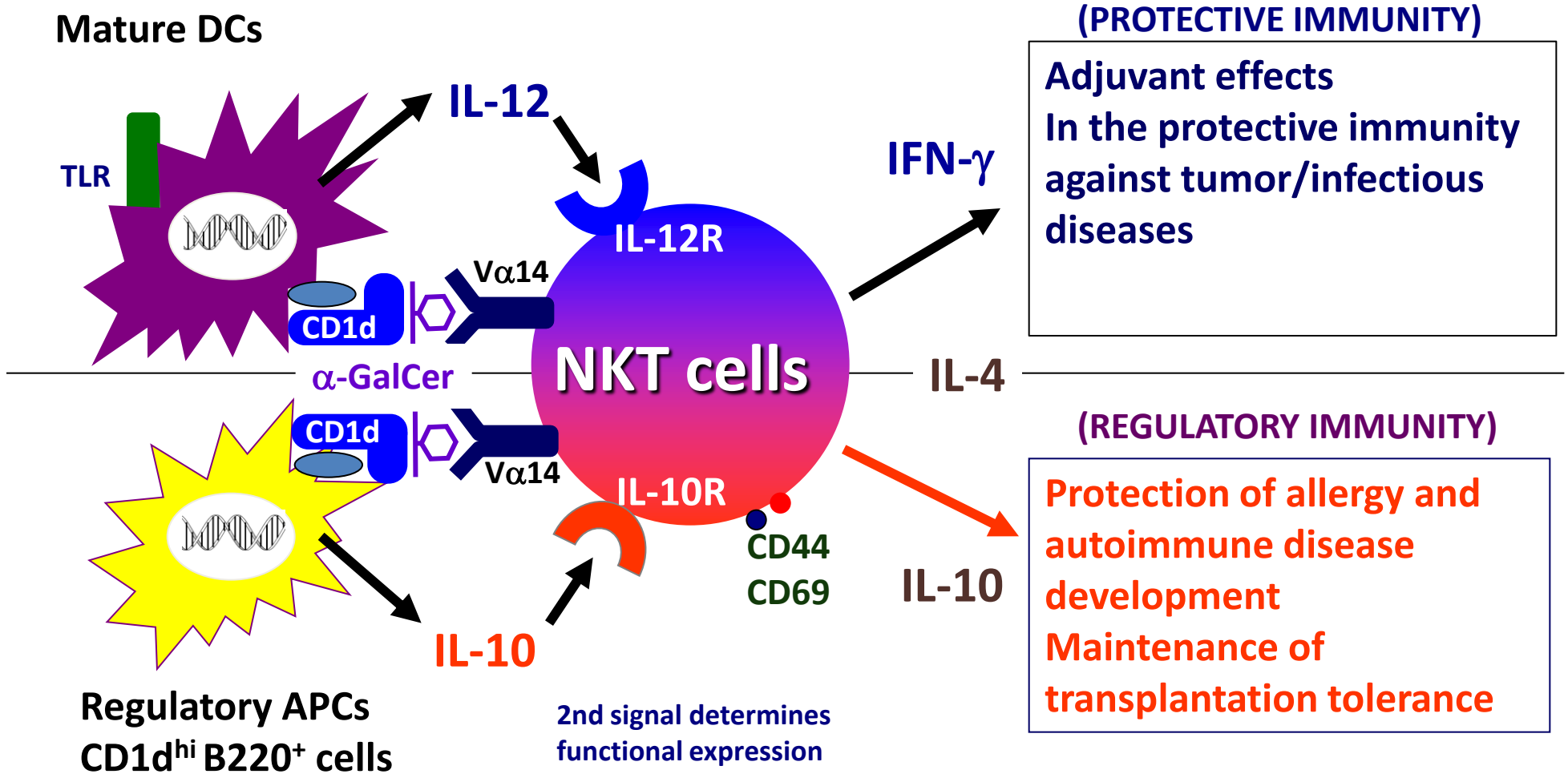




2001: Sharif S, Arrenza GA, Zucker P, et al. “Activation of natural killer T cells by α-galactosylceramide treatment prevents the onset and recurrence of autoimmune type 1 diabetes.” *Nat Med,* 7:1057.

iNKT細胞の多彩な機能は α-GalCerを提示する APCsによって制御されている

Innate Immune system — NKT system — Adaptive Immune system



α-GalCerアジュバントの臨床応用でのポイント

- α-GalCerを標的の抗原提示細胞にデリバーする
  - 投与ルート
  - 薬剤処方

KRN7000単独IV連投ではIFN-γは上昇しない

- 第I相臨床試験で癌患者さんにKRN7000を3回IV投与したが血中のIFN-γ の十分な上昇が認めらなかった。

Drug Formulation and Administration. The clinical dosage form of KRN7000 was supplied as vials containing lyophilized KRN7000 (0.2 mg), sucrose (56 mg), L-histidine (7.5 mg), and polysorbate 20 (5 mg) and was stored at 2°C to 8°C. Just before administration, KRN7000 was reconstituted with 1 ml of water. KRN7000 was administered by slow i.v. infusion on days 1, 8, and 15 of a 4-weekly cycle. Before drug administration, signs and symptoms of toxicity had to have disappeared. Doses administered and number of patients included at each dose level are summarized in Table 1.

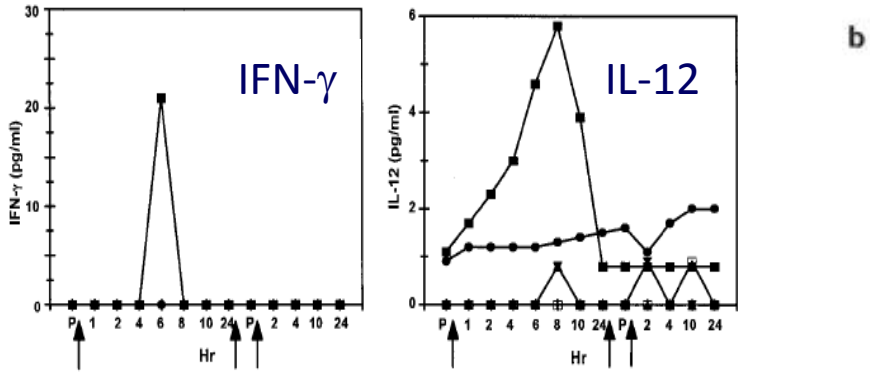
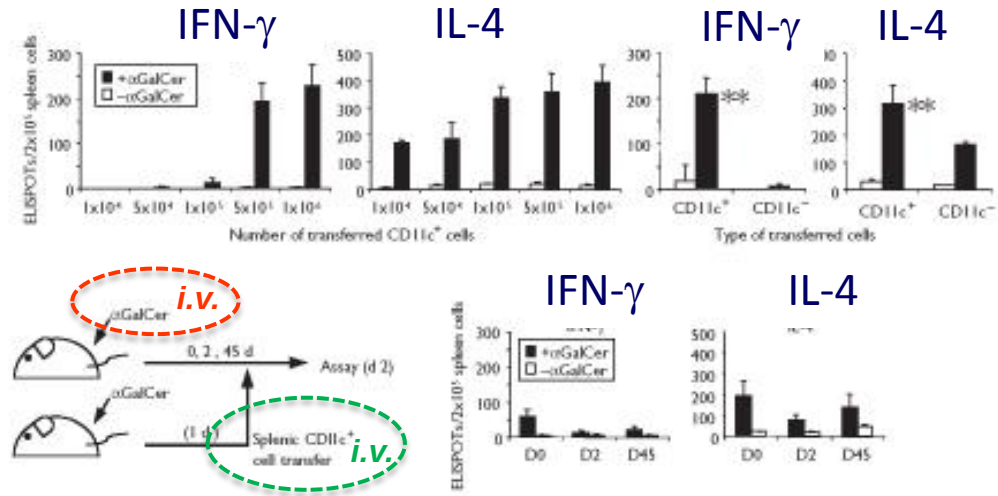


Fig. 4 Serum IFN-γ (*A*) and IL-12 (*B*) in patients with high pretreatment NKT-cell numbers (>333/ml; $n = 10$). Serum samples were collected before the first administration; at 1, 2, 4, 8, 10, and 24 h after the first administration; and on day 15 (before the third administration); and at 2, 4, 10, and 24 h after administration. Data represent the means of duplicate ELISA assays. Patient 3 showed an increase of both IFN-γ and IL-12 levels after the first administration. *Closed symbols*, the four patients with TNF-α and GM-CSF responses on KRN7000 (■, patient 3; ▲, patient 5; ●, patient 9; ▼, patient 17). *Arrows*, the three first weekly injections.

- 2002: Giaccone G, Punt C JA, Ando Y, et al. “A phase I study of the natural killer T-cell ligand α-galactosylceramide (KRN7000) in patients with solid tumors.” *Clin Cancer Res*, 8:3702.

- KRN7000を単独でIV投与した後に、KRN7000パルスしたDCをIV投与するとIFN-γ の産生が認められない。
  - KRN7000単独のIV投与で、NKT細胞の免疫抑制機能、たとえばIL-10産生が増強される可能性が示唆される。



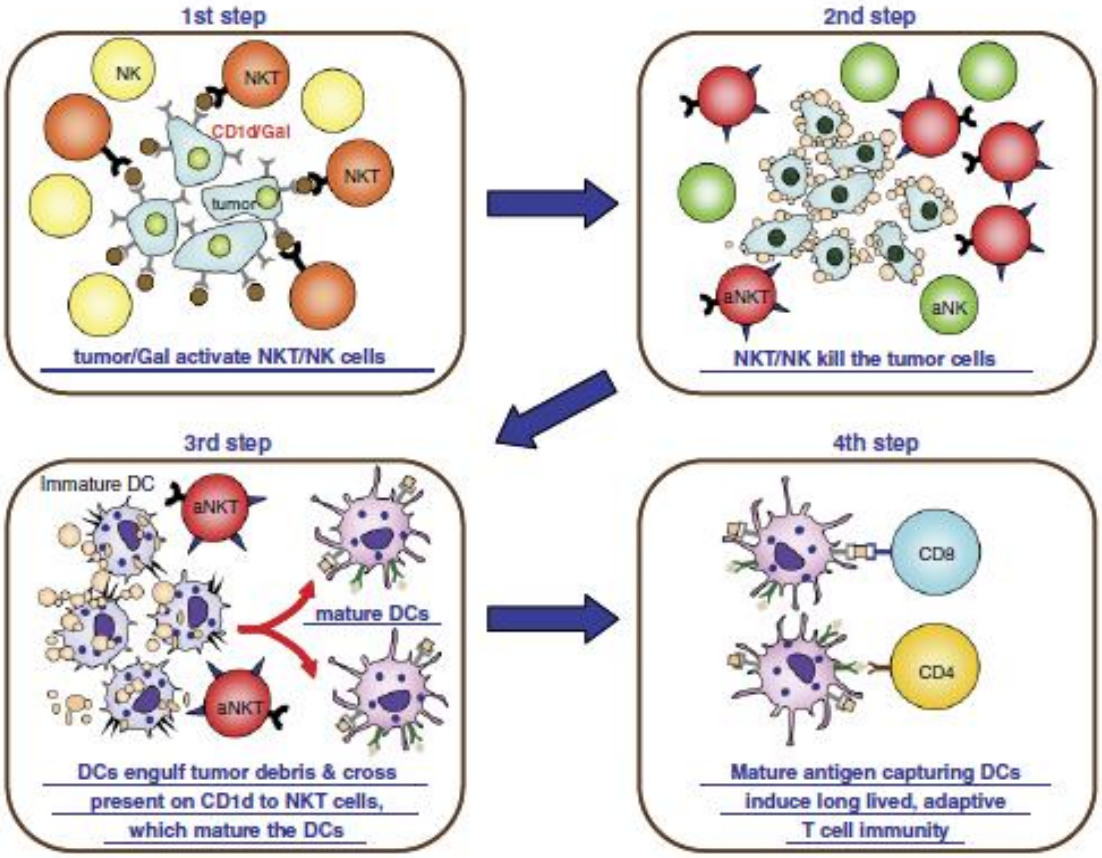
- 2002: Fuji S, Shimizu K, Kronenberg M, et al, “Prolonged IFN-g-producing NKT response induced with α-galactosylceramide-loaded DCs.” *Nat Immunol*, 3:867.

α-GalCerを使った癌ワクチン

- α-GalCerを樹状細胞にデリバーして、NKT細胞からのIFN-γ産生を誘導する
  - 投与ルート
    - 体外での細胞パルス
    - 静脈
  - 薬剤処方
    - 細胞
      - 患者末梢血細胞⇒ex vivo DC differentiation
      - 患者癌細胞
      - 人工アジュバントベクター細胞
    - ナノ粒子

がんワクチンへの応用

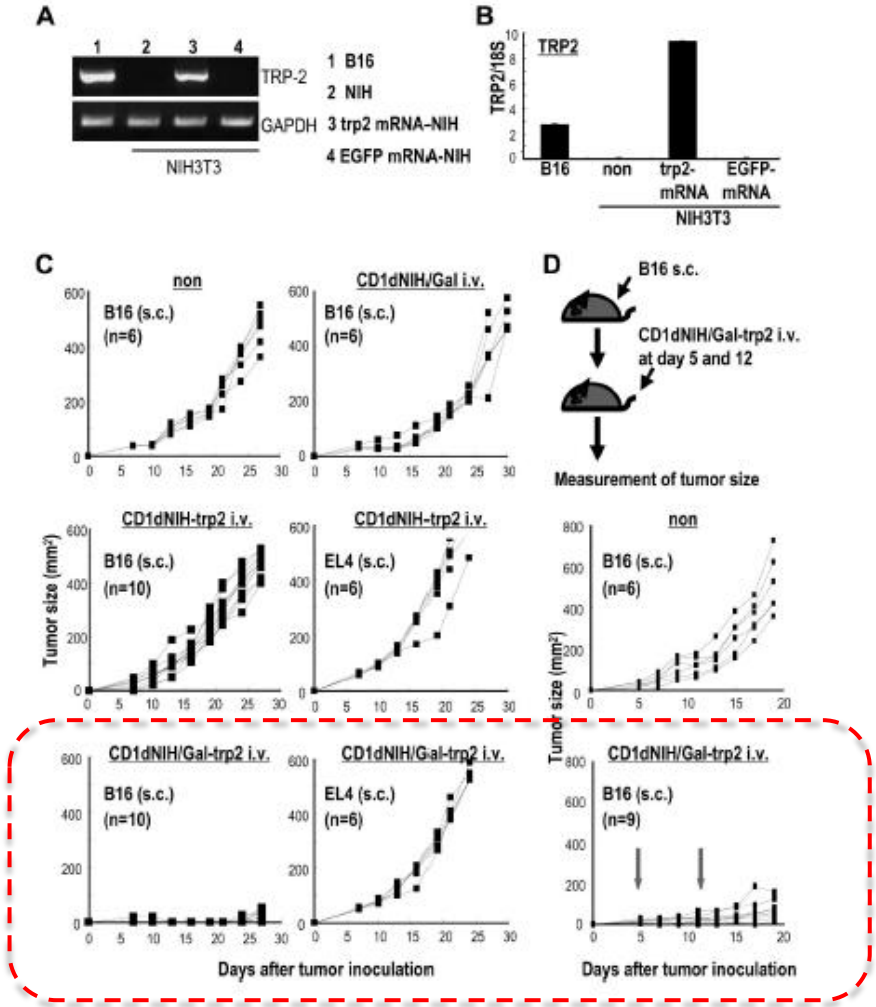
- がん細胞自身をベクターにするワクチン
  - α-GalCer (KRN7000)を取り込ませたB16メラノーマを体内に戻すとT細胞依存的な抗ガン作用を示す。



Fujii, S et al. 2007 *Immunol Rev* 220:183-

- 2007: Shimizu K, Kurosawa Y, Taniguchi M, Steinman RM and Fujii S, “Cross-presentation of glycolipid from tumor cells loaded with α-galactosylceramide leads to potent and long-lived T cell-mediated immunity via dendritic cells.” *J Exp Med*, 204:2641.

- 人工ベクター細胞を用いるワクチン
  - α-GalCer (KRN7000)とがん抗原mRNAを取り込ませたアロ繊維芽細胞を体内に戻すとT細胞依存的な抗ガン作用を示す。

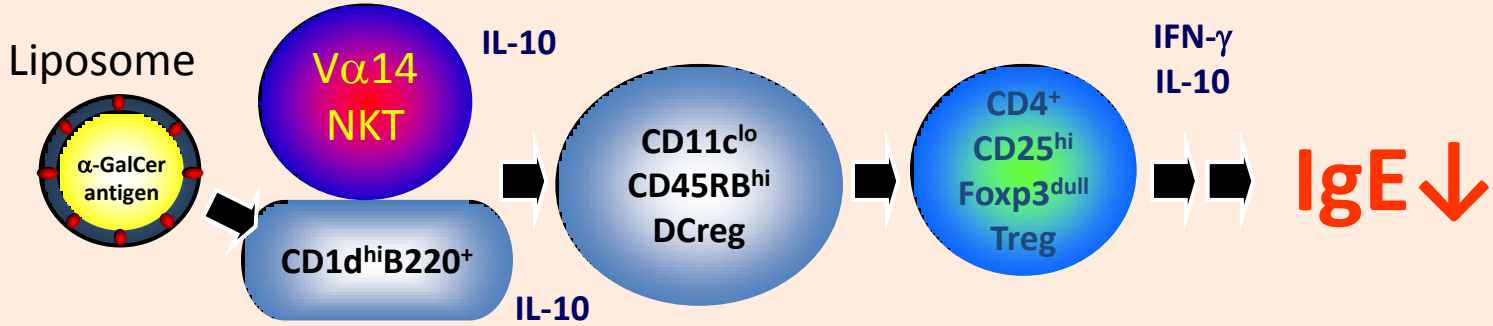


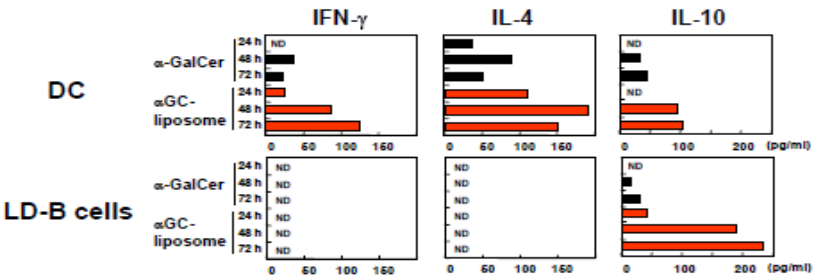
- 2009: Fuji S, Goto A and Shimizu K, “Antigen mRNA-transfected, allogenic fibroblasts loaded with NKT-cell ligand confer antitumor immunity.” *Blood*, 113:4262.

α-GalCerを使った免疫抑制ワクチン

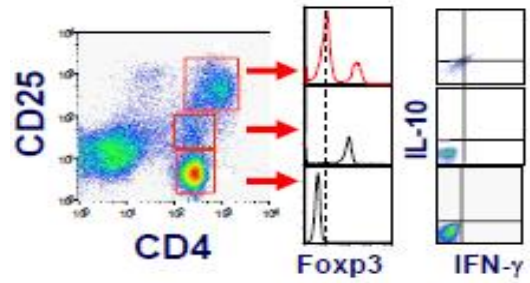
- α-GalCerをCD1d^{hi}-B細胞にデリバーして、NKT細胞からIL-10 産生を誘導し、Treg細胞分化を促進する。
  - 投与ルート
    - 静脈
      - 脾臓
    - 経口
  - 薬剤処方
    - ナノ粒子
      - リポソーム
    - 腸溶カプセル

アレルギーワクチンへの応用

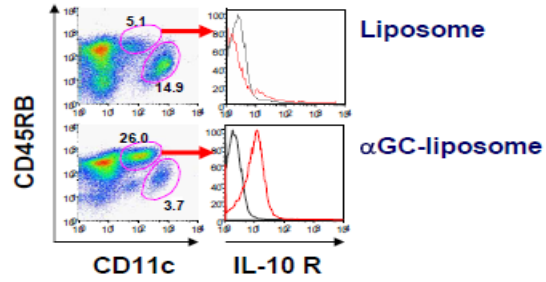




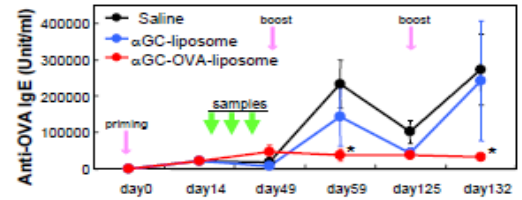
α-GalCerリポソームは脾臓.B220+細胞に取り込まれ、NKT細胞との会合によりIL-10産生が誘導される



IL-10とIFN-γを産生するCD4+CD25highFoxp3+T cellsが誘導される



IL-10Rを発現するCD11c^loCD45RB^hi が誘導される



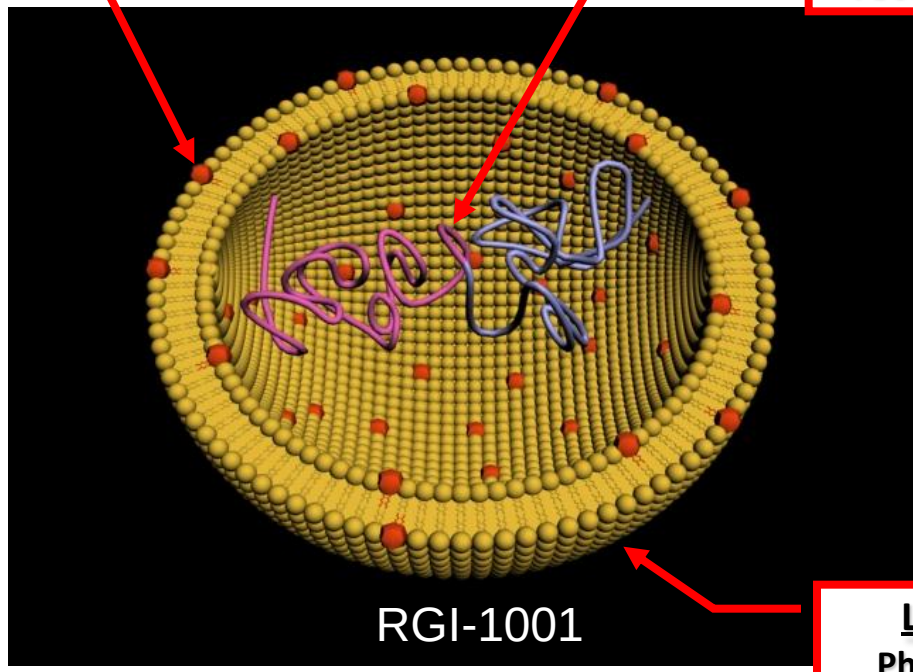
Ishii *et al*, 2008 *Front. Biosci.*

JST 独創的シーズ展開事業 革新的ベンチャー活用開発
「創薬イノベーション プログラム」

スギ花粉症ワクチン（RGI-1001）
-preclinical stage-

iNKT ligand
α-GalCer

Antigen
recombinant Cry j 1/2



Liposome
Phospholipids
Cholesterol

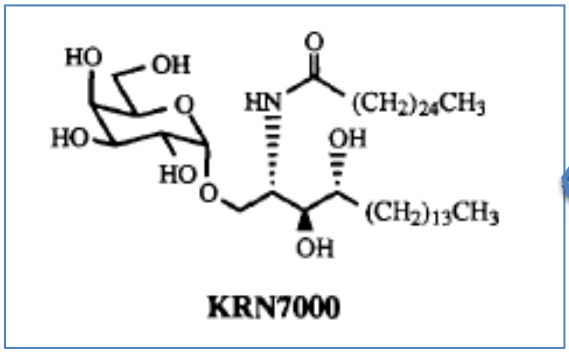
REGIMMUNE

α-GalCerを使った感染症ワクチン

- α-GalCerを粘膜下の樹状細胞にデリバーして、NKT細胞からのIL-4 産生を誘導し、IgA産生を促進する。
  - 投与ルート
    - 経鼻
      - NALT
    - 経口
      - GALT
  - 薬剤処方
    - ナノ粒子
      - リポソーム
    - 腸溶カプセル

REGIMMUNE

α-GalCerアジュバントの臨床開発ポテンシャル



腸管粘膜
CD1d^{hi}
B220^{+}

気道粘膜
DC

脾臓
DC
iNKT
→ がん
感染症

脾臓
CD1d^{hi}
B220^{+}
iNKT
→ アレルギー
自己免疫疾患
移植拒絶

鼻粘膜
DC
iNKT
→ 感染症（インフルエンザ）

腸管粘膜
DC
iNKT
→ 炎症性腸疾患
アレルギー