The Affect and Effect of IL18

Reflections of an Unreconstructed Tumor Immunologist

Society for Immunotherapy of Cancer

Surgery Branch, NCI, August 29, 2001

Michael T. Lotze, MD, FACS

Education SITC (MTL: Nurix Therapeutics)

July 18, 2022

11:30-2pm CDT

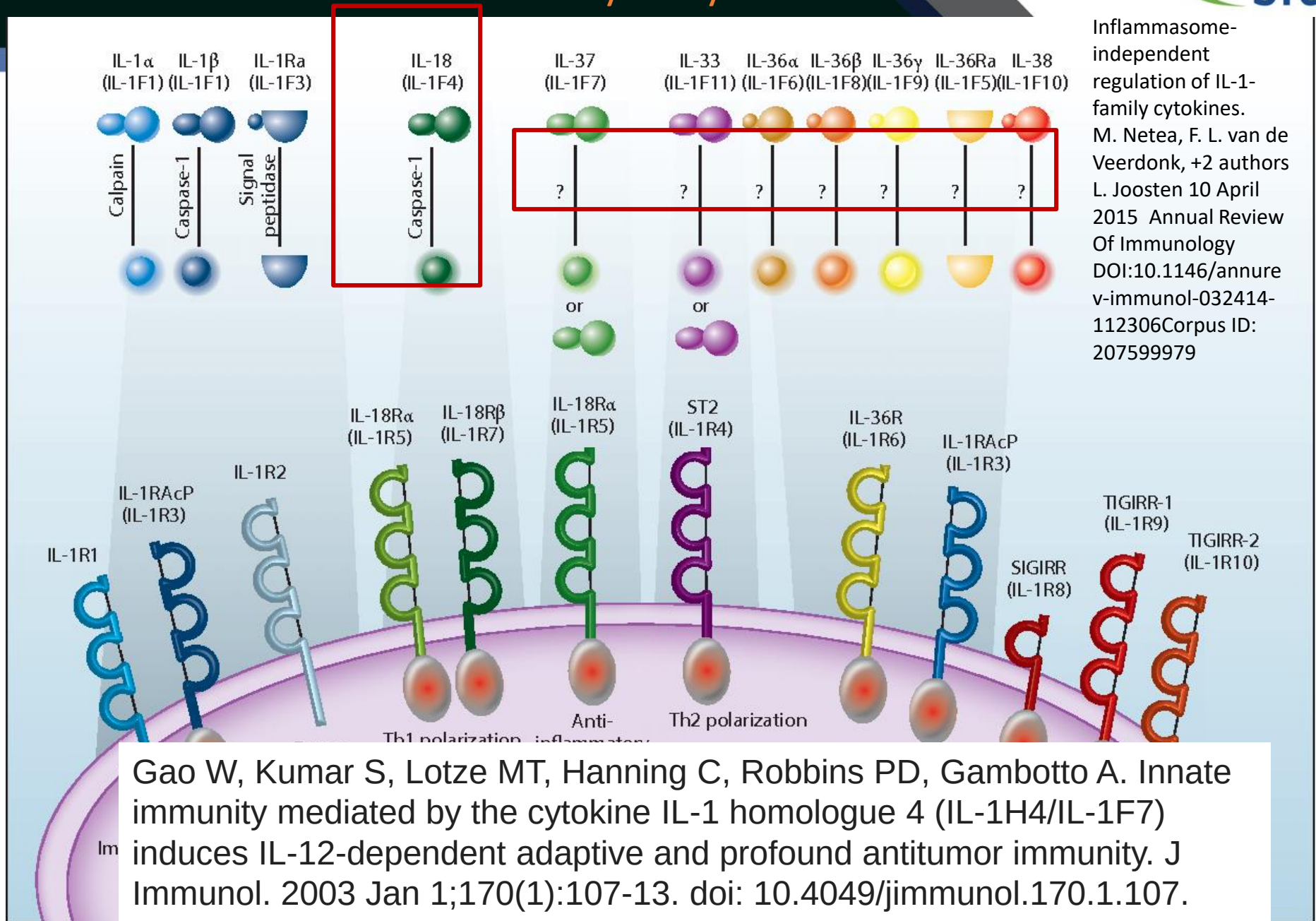
nurix

Targets for Cancer IO: A Deep Dive, Recombinant Interleukin 18 Immunotherapy

Competing Interests

Nurix Therapeutics, Chief Cell Therapy Officer (CCO)
Checkmate, Advisor
iRepertoire, Advisor

Interleukin-18 is a Member of the IL-1 Family of Cytokines



Inflammasome-independent regulation of IL-1-family cytokines. M. Netea, F. L. van de Veerdonk, +2 authors L. Joosten 10 April 2015 Annual Review Of Immunology DOI:10.1146/annurev-immunol-032414-112306Corpus ID: 207599979

Gao W, Kumar S, Lotze MT, Hanning C, Robbins PD, Gambotto A. Innate immunity mediated by the cytokine IL-1 homologue 4 (IL-1H4/IL-1F7) induces IL-12-dependent adaptive and profound antitumor immunity. J Immunol. 2003 Jan 1;170(1):107-13. doi: 10.4049/jimmunol.170.1.107.

Martin SJ, Frezza V, Davidovich P, Najda Z, Clancy DM. IL-1 family cytokines serve as 'activity recognition receptors' for aberrant protease activity indicative of danger. Cytokine. 2022 Jun 24;157:155935.

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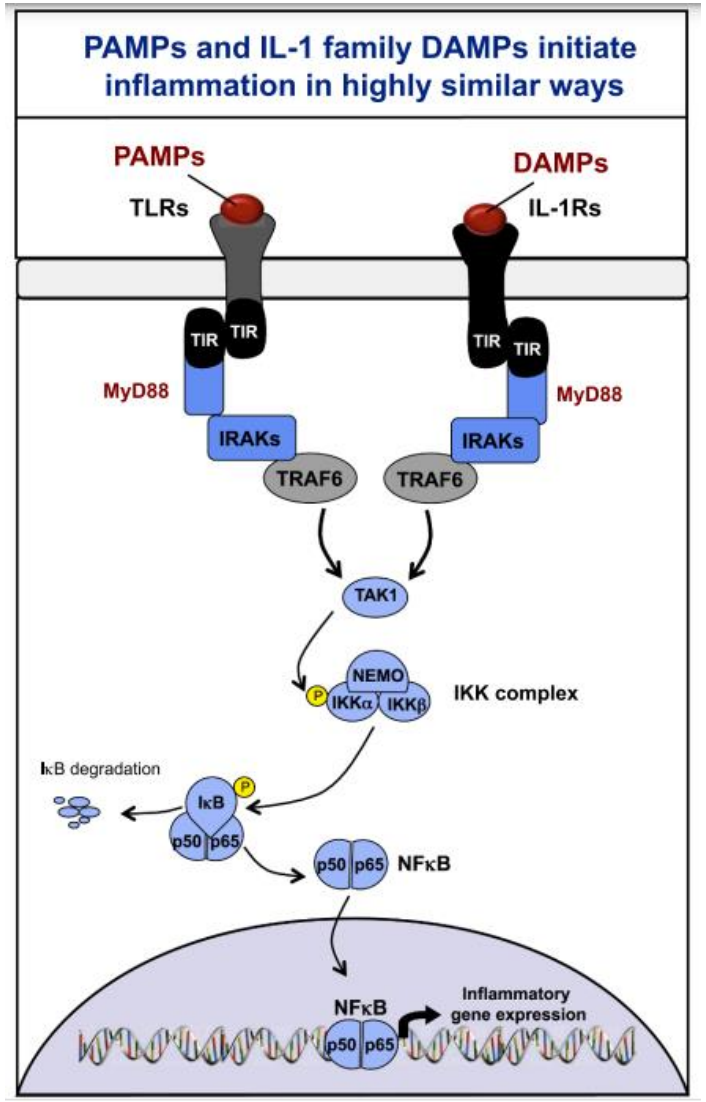

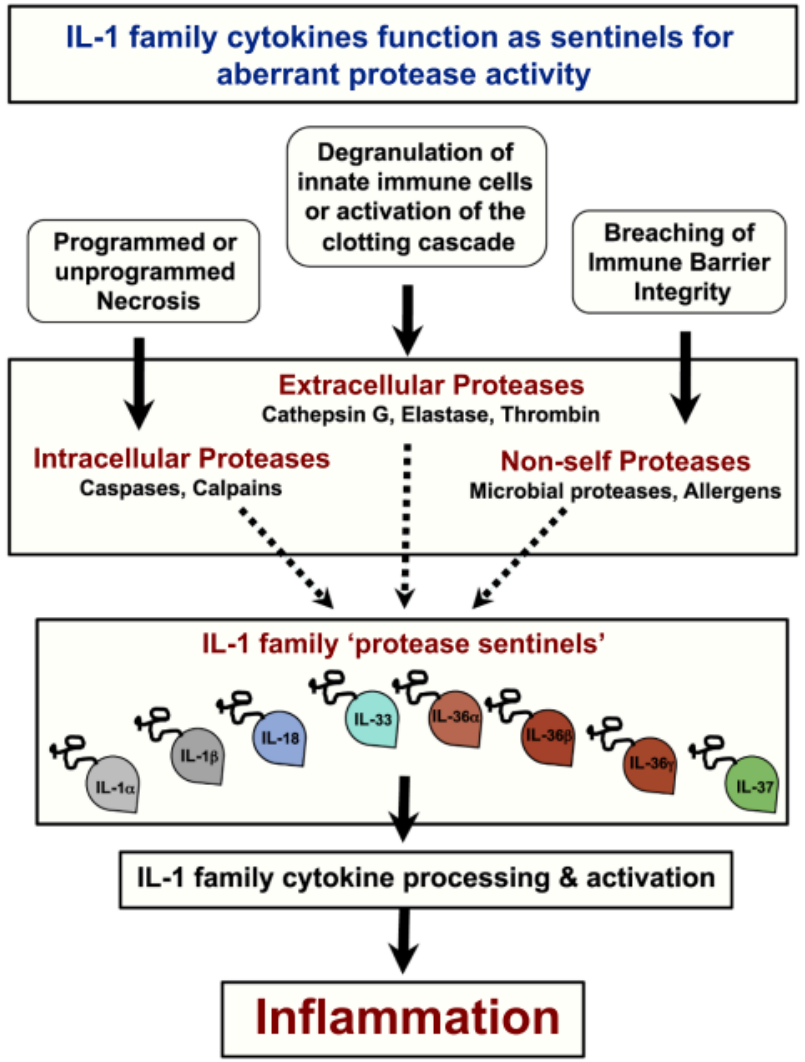

Interleukin 18: Discovery

- *OKAMURA.* A NOVEL COSTIMULATORY FACTOR FOR γ INTERFERON INDUCTION FOUND IN THE LIVER OF MICE CAUSES ENDOTOXIC SHOCK. *INFECT. IMMUN. 63:3966, 1995.*
- *OKAMURA, NAKANISHI (Hyogo).* CLONING OF A NEW CYTOKINE THAT INDUCES IFN γ PRODUCTION BY T CELLS. *NATURE 378:88, 1995.*
- *USHIO.*CLONING OF THE cDNA FOR HUMAN IFN- γ INDUCING FACTOR, EXPRESSION *E. COLI*, AND STUDIES ON THE BIOLOGIC ACTIVITIES OF THE PROTEIN. *J IMMUN. 156:4274, 1996.*
- *MICALIEF.* INTERFERON-GAMMA-INDUCING FACTOR ENHANCES T HELPER 1 CYTOKINE PRODUCTION BY STIMULATED HUMAN T CELLS: SYNERGISM WITH IL12 FOR INTERFERON- γ PRODUCTION. *EUR. J. IMMUN. 26:1647, 1996.*

IL-18
(Interferon-Gamma-Inducing Factor)

- Induces IL1 alpha, IL8, GM-CSF, and IFN-γ production from T- and NK cells.
- Has nucleotide homology with IL-1α (12 %), IL-1β (19 %), IL-1RA, and the other IL-1 Family Members
- Lacks a typical signal sequence for cytokines and is cleaved to a mature (biologically active) form by Caspase-1 (IL-1 β converting enzyme- ICE) or Caspase 4.

Amino acid sequence of human IL-18 and homology with IL-1β

Sequence alignment below from S. Kumar, et al., *J. Biol. Chem.*, 275, 10308-10314, 2000.
See also J.F. Bazan, et al., *Nature*, 379, 591, 1996.

Approximately 33 % similarity with IL-β

```
hIL-18         1  YFGKLES KLSVIRNLND QVLFIDQGNR PLFEDMTDSD CRDNAPRTIF
hIL-1beta      1    APVRS LNCTLRDSQQ KSLVMSGPYE LKALHLQGQD MEQQVVFSM-
                     . . *     .*.  .   * .           .   *   .    ..

hIL-18        48  IISMYKDSQP RGMAVTISVK CEKISTLSCE -NKIIS-FKE
MNPPDNIKDT
hIL-1beta     45  --SFVQGEES NDKIPVALGL KEKNLYLSCV LKDD--KPTL
QLESVDPKNY
                    *     ..   .         **   ***   .            . . *.

hIL-18        96  K---SDIIFF QRSVPGHDNK MQFESSSYEG YFLACEKERD L-
FKLILKKE
hIL-1beta     91  PKKKMEKRFV FNKIEI-NNK LEFESAQFPN WYISTSQAEN
MPVFLGGTKG
                        .  *     .   .** ..***. .   ...      . .   *   *

hIL-18       142  DELGDRSIMF TVQNED
hIL-1beta    140  ---GQDITDF TMQFVS
                     *.    * *.*
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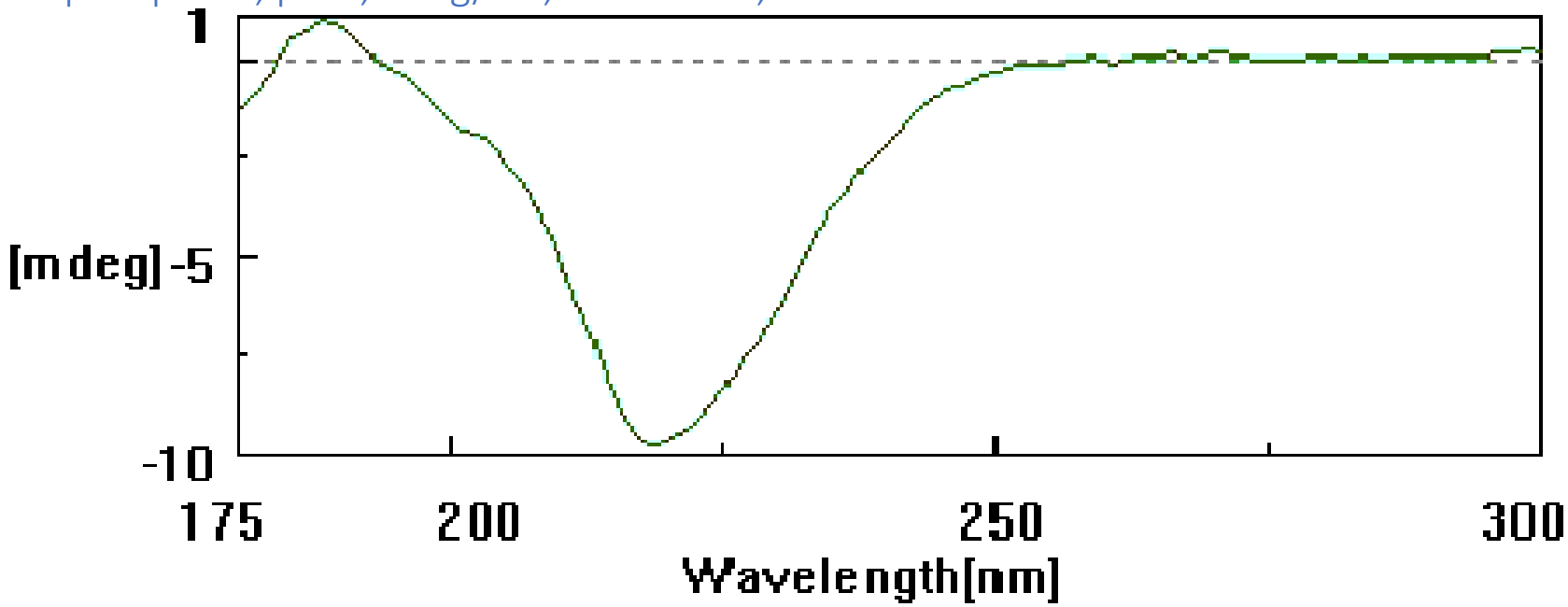
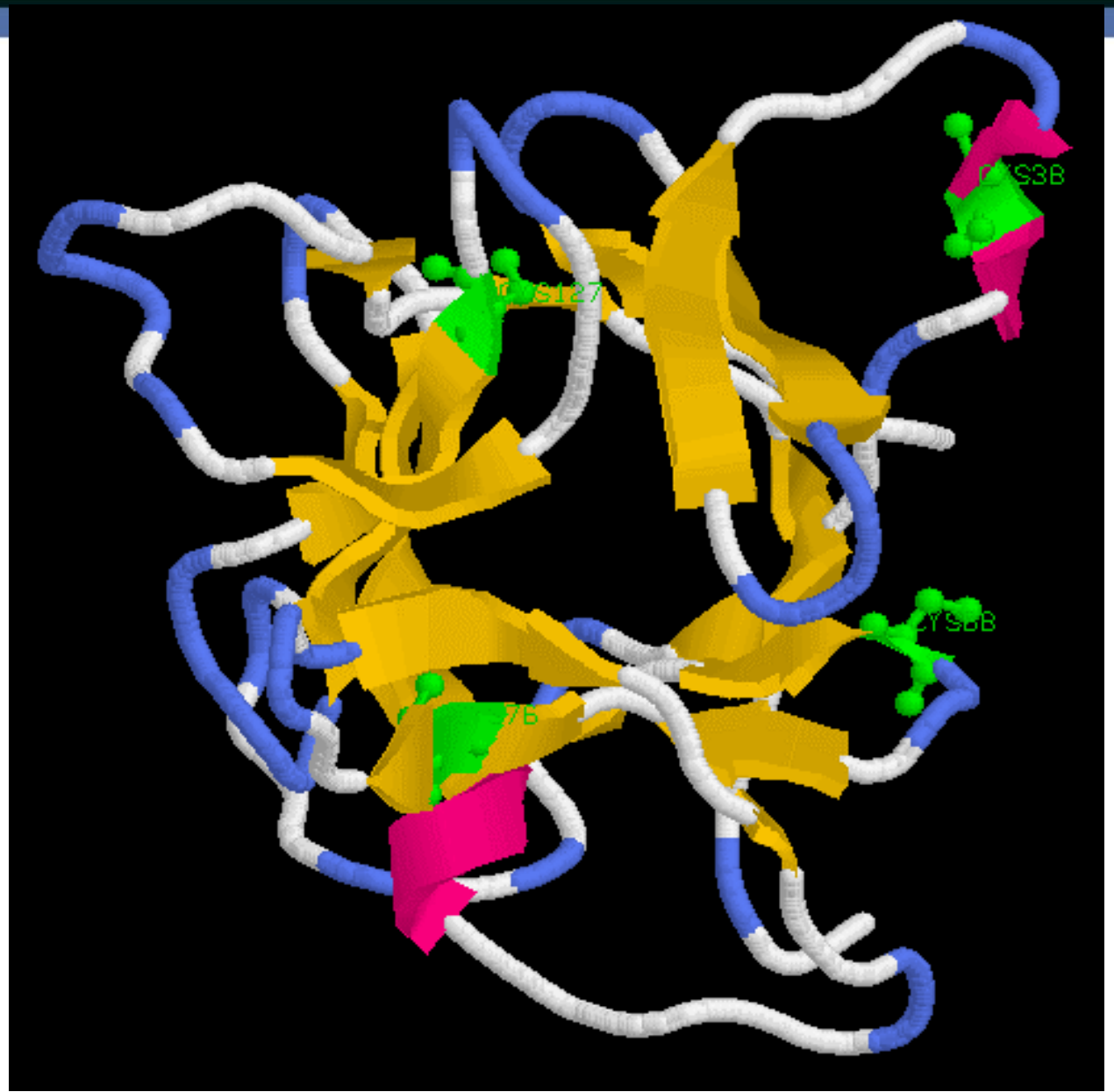
Circular dichroism spectrum of recombinant human IL-18 (D. Cronin)

5 mM sodium phosphate, pH 7, 1 mg/mL, 0.5 cm cell, Jasco J-810



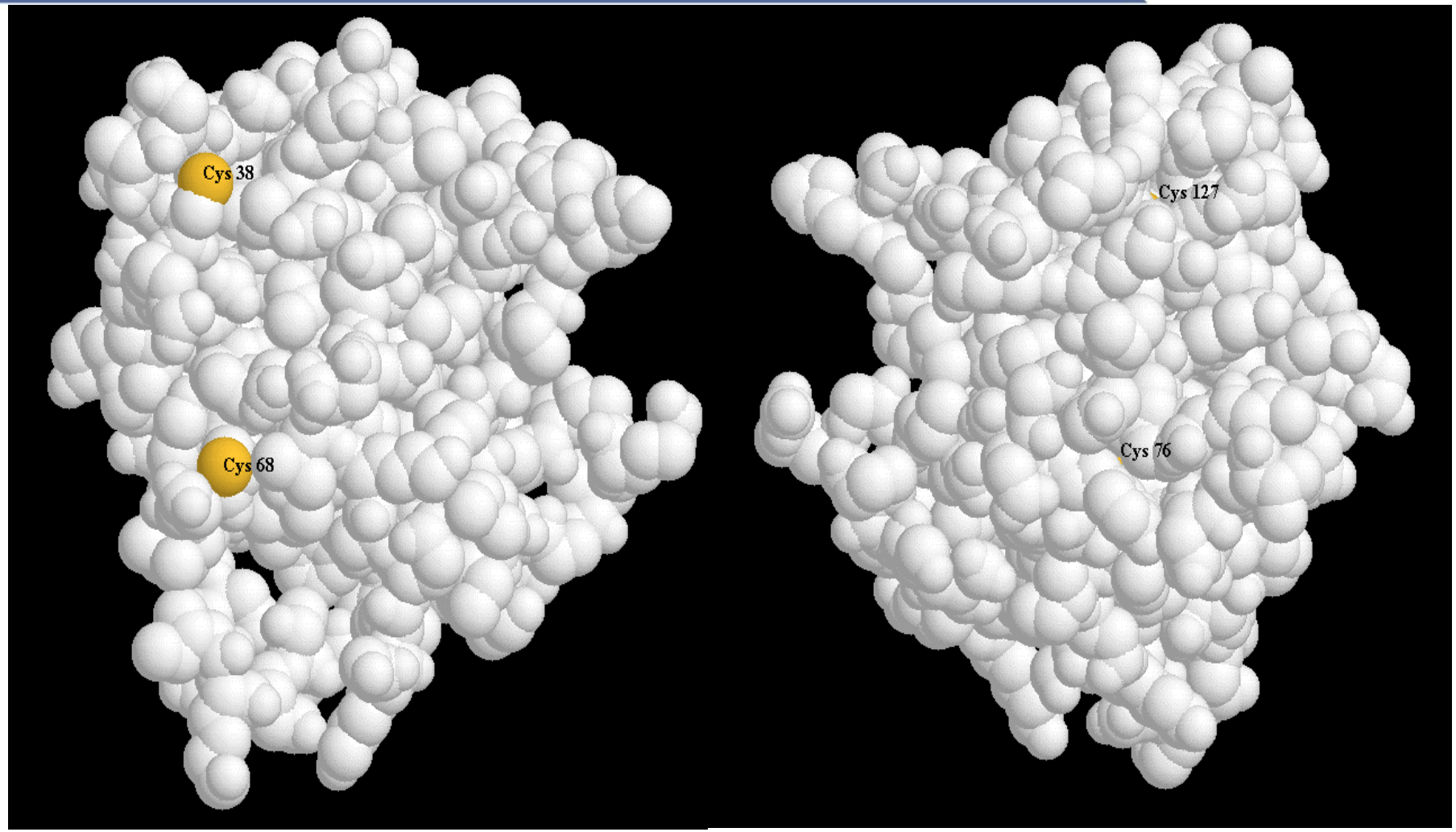
Spectrum is similar to that reported for human IL-1β and other proteins with a high content of beta-sheet secondary structure.

Swiss-Model of human IL-18



These cytokines all possess a conserved β-trefoil conformation and a central hydrophobic core composed of 12 β-sheets, six of which (β1, β4, β5, β8, β9, and β12) form an antiparallel β-barrel.

Solvent exposures of cysteines in IL-18 homology model

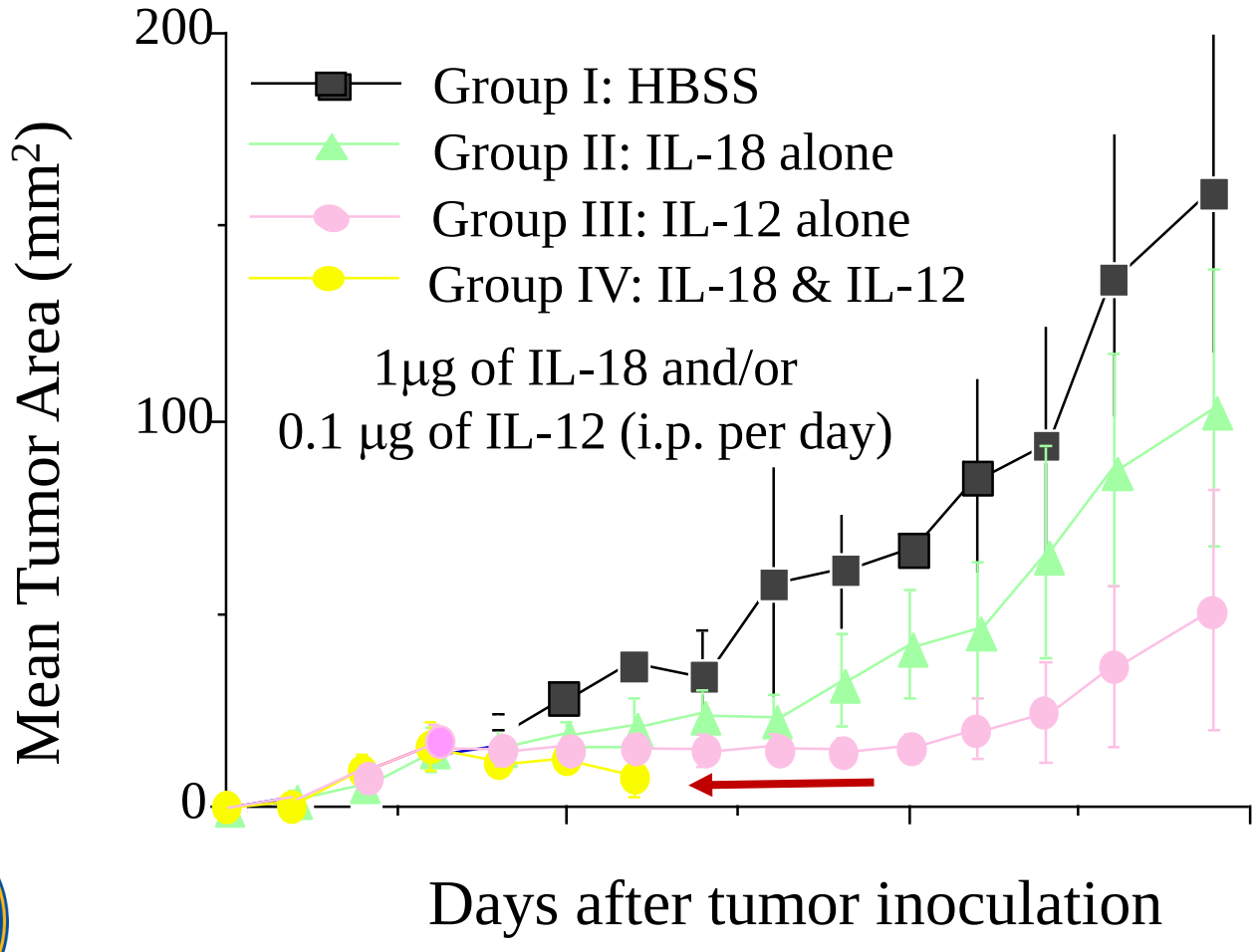


Medical Sciences: IL-18 binding and inhibition of interferon induction by human poxvirus-encoded proteins

Yan Xiang and Bernard Moss PNAS 96:11537-11542, September 28, 1999

Molluscum contagiosum virus (MCV) is a common, human poxvirus that causes small papular skin lesions that persist for long periods without signs of inflammation. Previous studies revealed that MCV encodes a family of proteins with homology to mammalian IL-18 binding proteins. IL-18 is a proinflammatory cytokine that induces synthesis of interferon , activates NK cells, and is required for a T-lymphocyte helper type 1 response. We expressed and purified the proteins encoded by the MC53L and MC54L genes of MCV, as well as their human and murine homologs. All four recombinant proteins were able to bind with high affinity to human and murine IL-18 molecules and inhibited IL-18 mediated interferon production in a dose-dependent manner. The pirating of IL-18 binding proteins by poxviruses and their use as decoy receptors is consistent with the critical role of IL-18 in defense against virus infections and provides a mechanism for evasion of the immune system by MCV.

Anti-tumor Effects of rIL-18 Combined with rIL-12 on MCA205



Osaki T, Péron JM, Cai Q, Okamura H, Robbins PD, Kurimoto M, Lotze MT, Tahara H. IFN-gamma-inducing factor/IL-18 administration mediates IFN-gamma- and IL-12-independent antitumor effects. J Immunol. 1998 Feb 15;160(4):1742-9. PMID: 9469432.

Marked Elevation of Serum IFN-γ Level With Systemic Administration of rIL-18 Combined with rIL-12

Treatment*	IFN-γ (Mean + SD) pg/ml	
	Before	5th day
HBSS	<15.6	<15.6
IL-18	<15.6	57.1 ± 64.9
IL-12	<15.6	400.0 ± 577.5
IL-18 and IL-12	<15.6	17,671.5 ± 107.1

*C57BL/6 mice received HBSS, IL-18 (1μg/day) or/and IL-12 (0.1μg/day)

Osaki T, Péron JM, Cai Q, Okamura H, Robbins PD, Kurimoto M, Lotze MT, Tahara H. IFN-gamma-inducing factor/IL-18 administration mediates IFN-gamma- and IL-12-independent antitumor effects. J Immunol. 1998 Feb 15;160(4):1742-9. PMID: 9469432.

Findings in rIL-18 protein studies (Anti-tumor effects)

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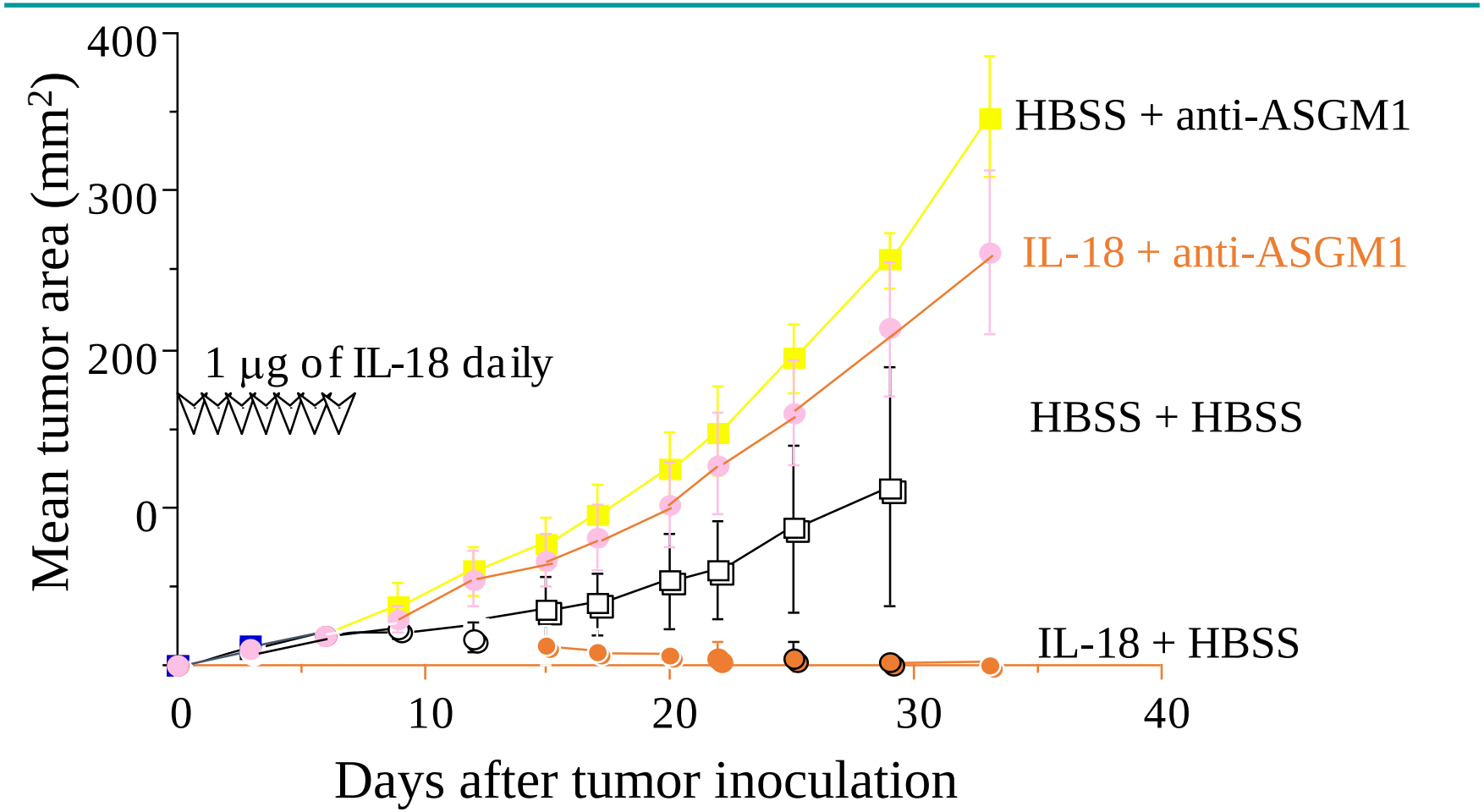
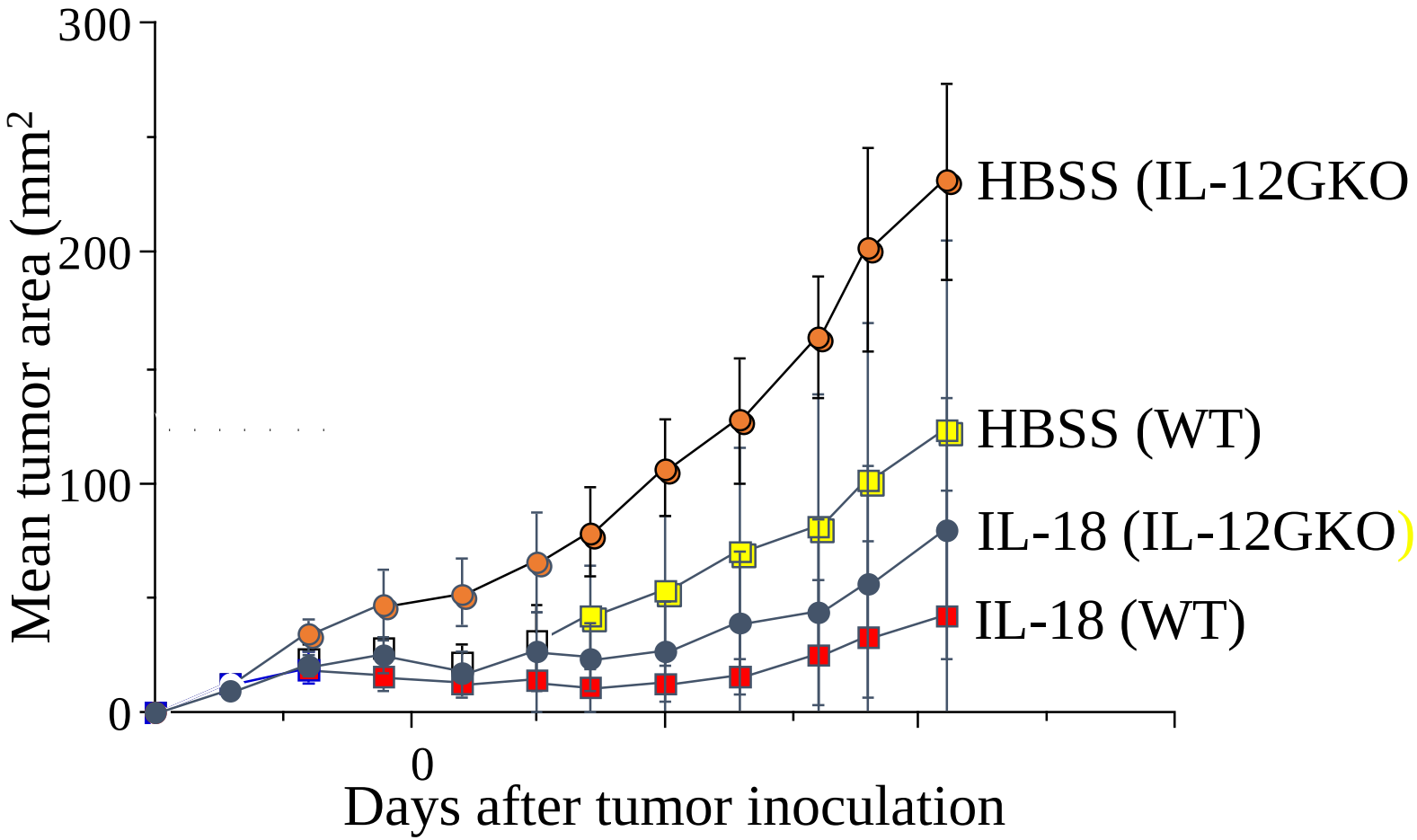
- IL-18 administration significantly suppresses the growth of murine i.d. tumors (CL8-1 melanoma, MCA205 sarcoma).
- Most animals become immune to the tumor after successful treatment.
- IL-18/IL-12 combination therapy has the most significant and immediate anti-tumor effects.
- However, many mice so treated succumb with markedly elevated serum IFN-γ levels.

Abrogation of Anti-tumor Effects of rIL-18 by Anti-ASGM-1 Administration (NK)



Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, Lotze MT, Tahara H. Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin-induced tumor apoptosis, respectively. J Immunol. 1999 Jul 15;163(2):583-9. PMID: 10395644.

Significant anti-tumor effects of rIL-18 in IL-12 gene disrupted mice



Findings in rIL-18 protein studies
(Effector cell populations and involved cytokines)

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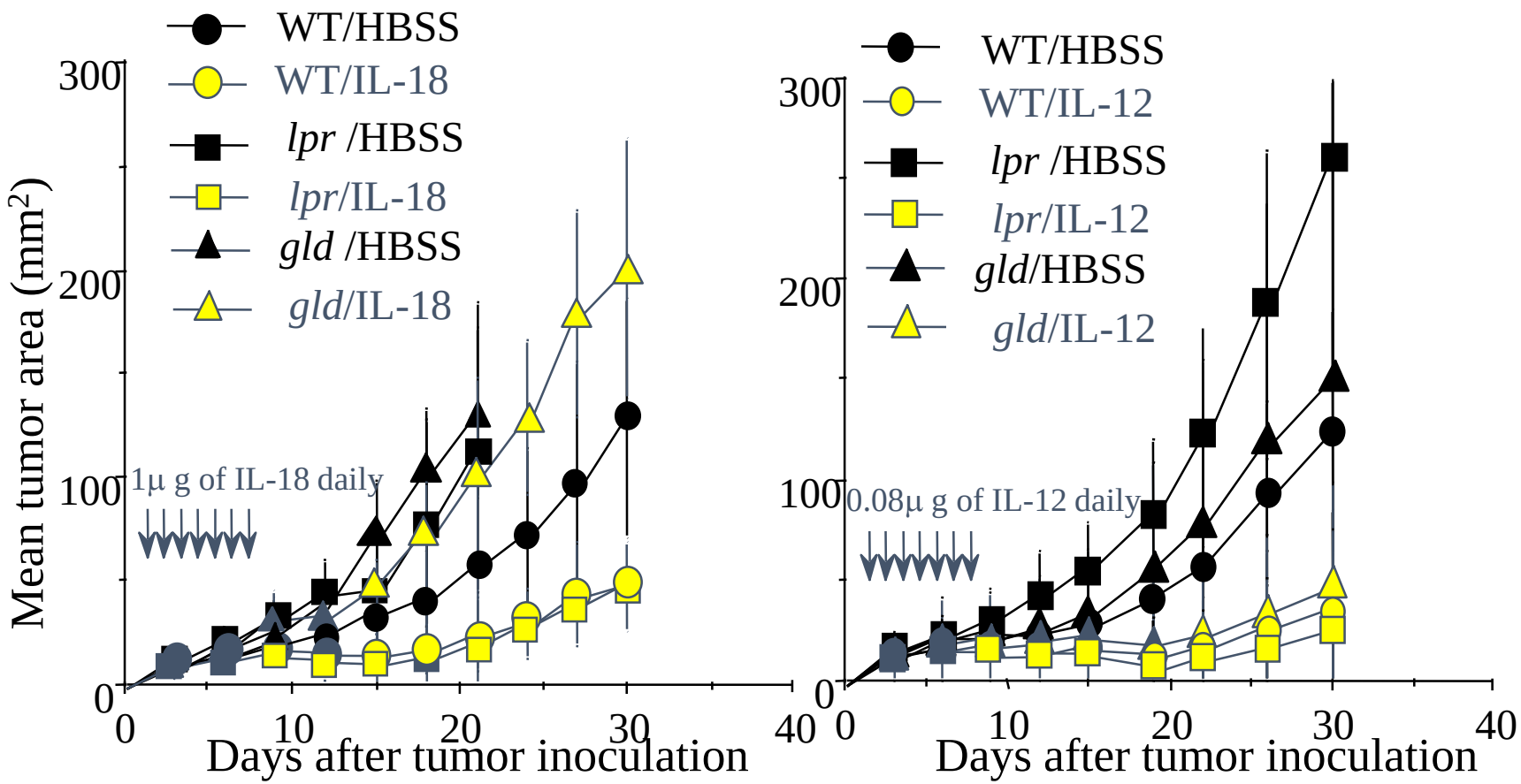
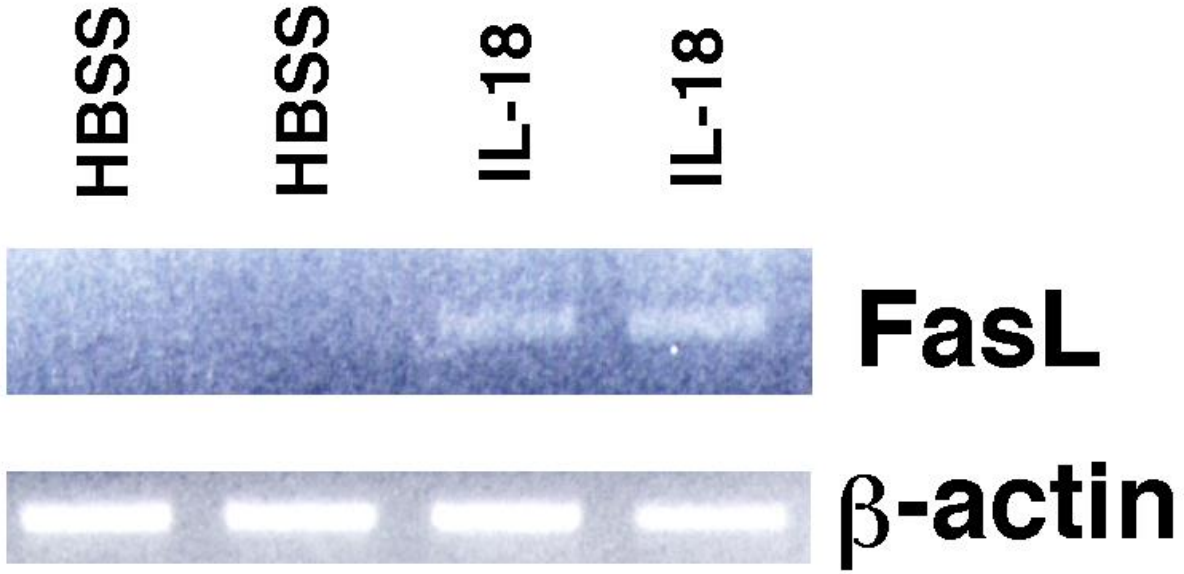
- Anti-tumor effects of rIL-18
  - completely abrogated with the administration of anti-asialo GM1 (ASGM1) antibody
  - only partially impaired in IFN-□ or IL-12 gene disrupted mice.
- Immunohistochemical examination of the tumors in animals treated with IL-18.
  - CD8+ T cells ; reduced number
  - CD4+ T cells ; no change

Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, Lotze MT, Tahara H. Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin-induced tumor apoptosis, respectively. J Immunol. 1999 Jul 15;163(2):583-9. PMID: 10395644.

Antitumor effects of IL-18, but not IL-12 Abrogated in Fas-L deficient *gld* mice



Expression of FasL mRNA on NK cells is enhanced following rIL-18 administration



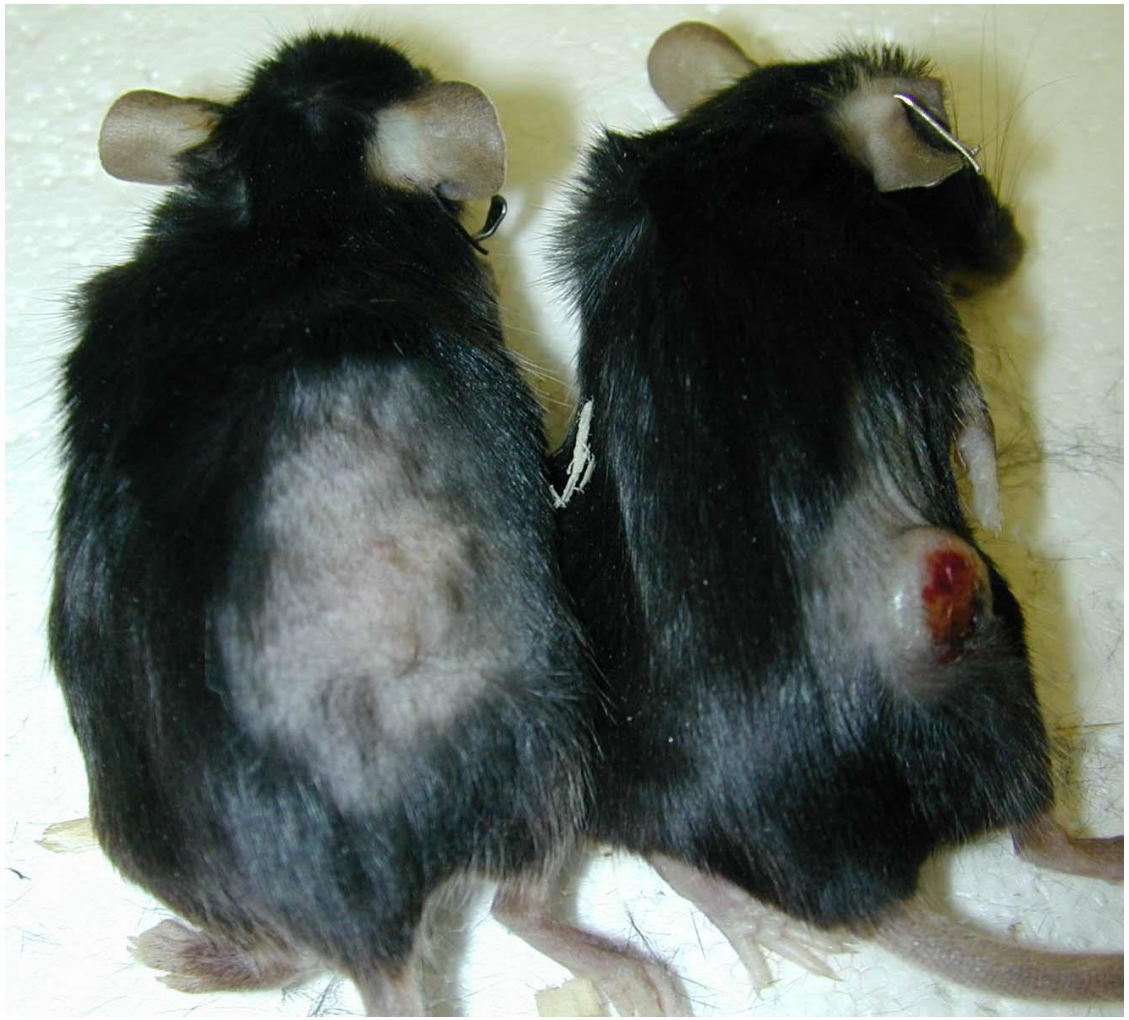
Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, Lotze MT, Tahara H. Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin-induced tumor apoptosis, respectively. J Immunol. 1999 Jul 15;163(2):583-9. PMID: 10395644.

Effects of IL-12 and IL-18 on innate effector mechanisms

		Apoptotic pathways		
	ASGM1+ cells	Perforin	Fas	IFN-γ
IL-12	+	+++	-	+++
IL-18	+++	+*	+++	+

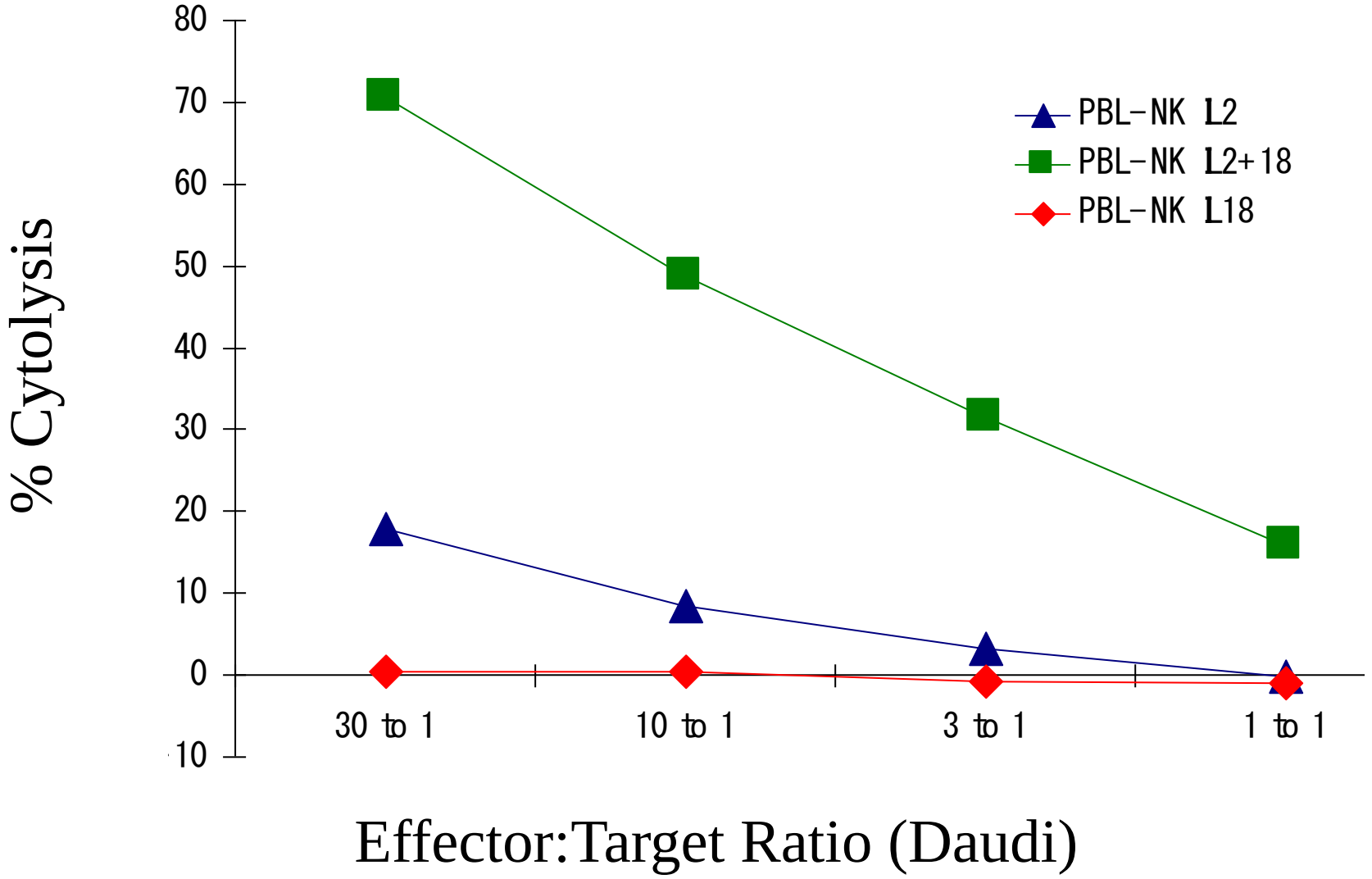
*: *In vitro* toxicity

Day 12 Treatment with IL2 and IL18
MC38 Colorectal Carcinoma

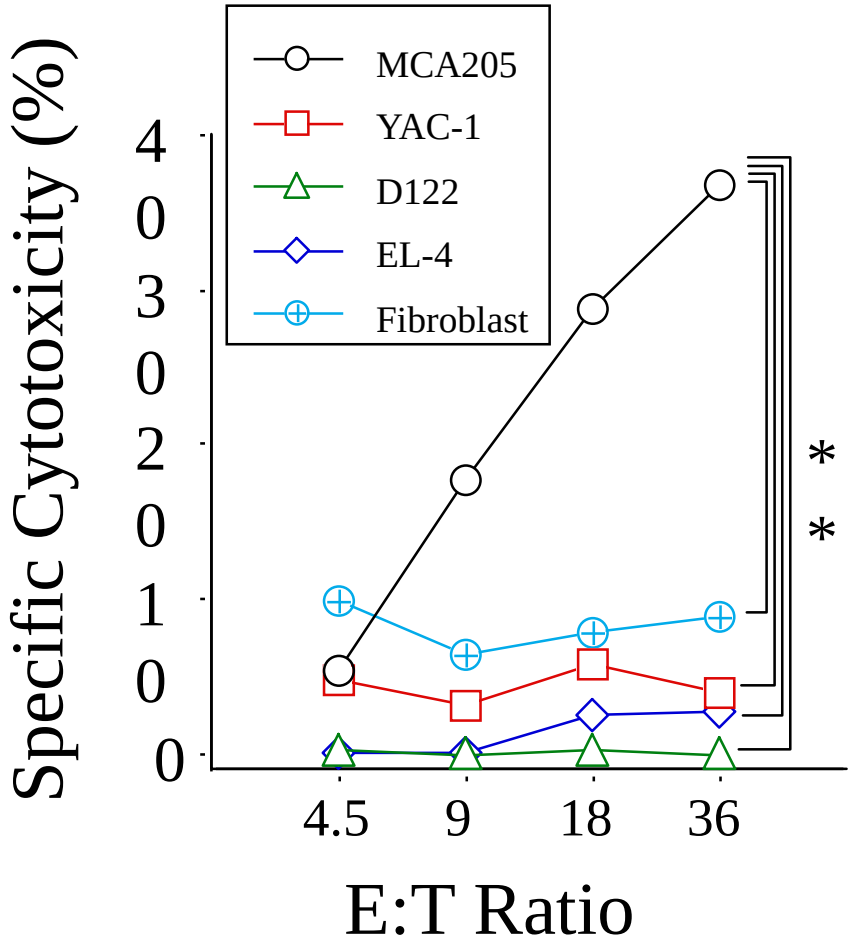


Son YI, Dallal RM, Mailliard RB, Egawa S, Jonak ZL, Lotze MT. Interleukin-18 (IL-18) synergizes with IL-2 to enhance cytotoxicity, interferon-gamma production, and expansion of natural killer cells. Cancer Res. 2001 Feb 1;61(3):884-8. PMID: 11221875

IL18 Enhances Cytolysis Mediated by Day 4 CD56+ Selected Cells Stimulated by IL2



Fine Specificity of D4 Effector Cells Obtained in Combined Coculture System-Yosenabe Culture

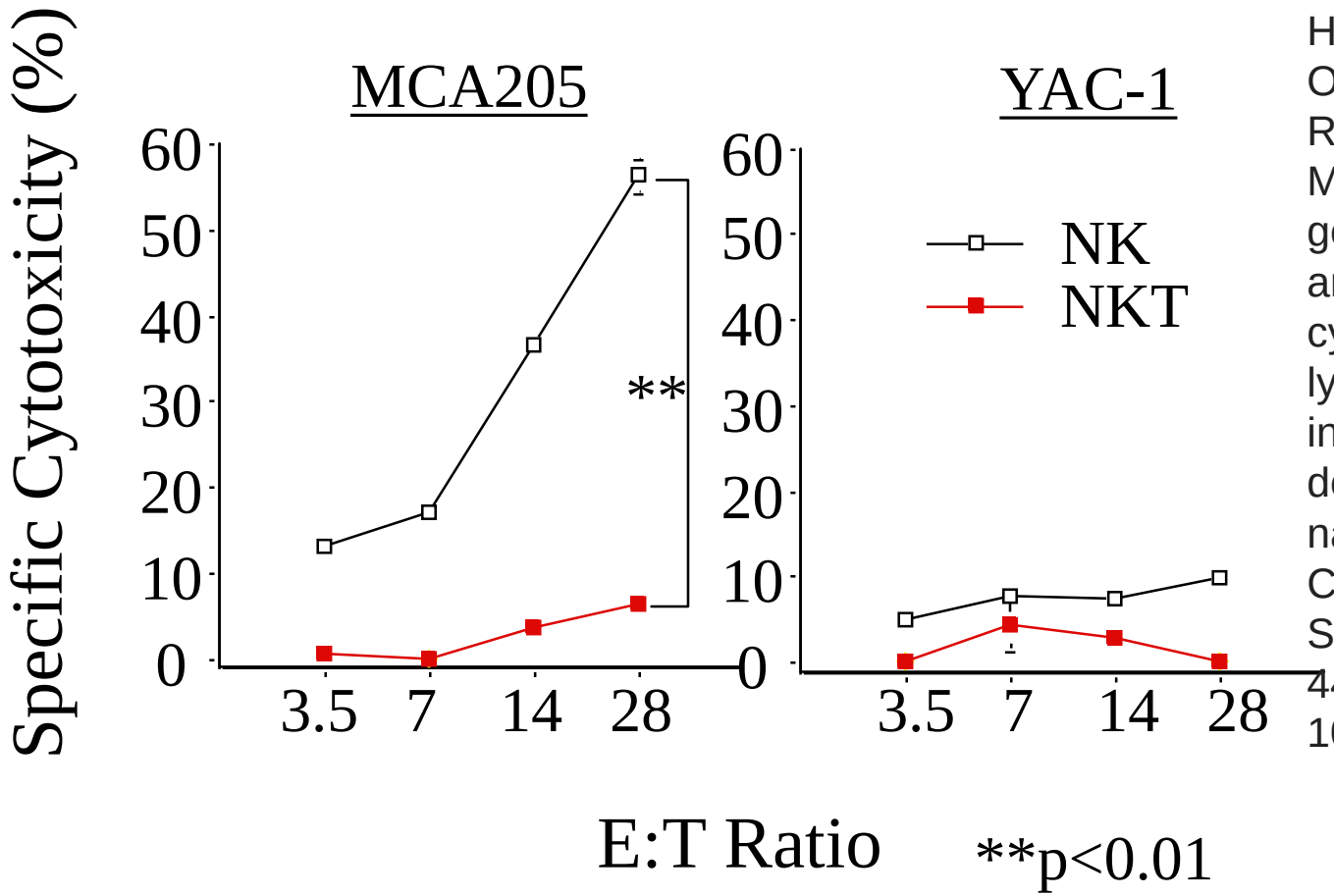


Tanaka F, Hashimoto W, Okamura H, Robbins PD, Lotze MT, Tahara H. Rapid generation of potent and tumor-specific cytotoxic T lymphocytes by interleukin 18 using dendritic cells and natural killer cells. Cancer Res. 2000 Sep 1;60(17):4838-44. PMID: 10987295.

**p<0.01

Splenic T cells naïve mice+MCA205+NK/IL-2+BM-DC+IL-18

Sorted NK1.1+/CD3- NK Cells, and not NK1.1+/CD3+ NKT Cells Critical Role in Generating CTL in Cooperative Coculture



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High-dose IL-18 Induces Regression of Advanced MOPC-315 Plasmacytoma

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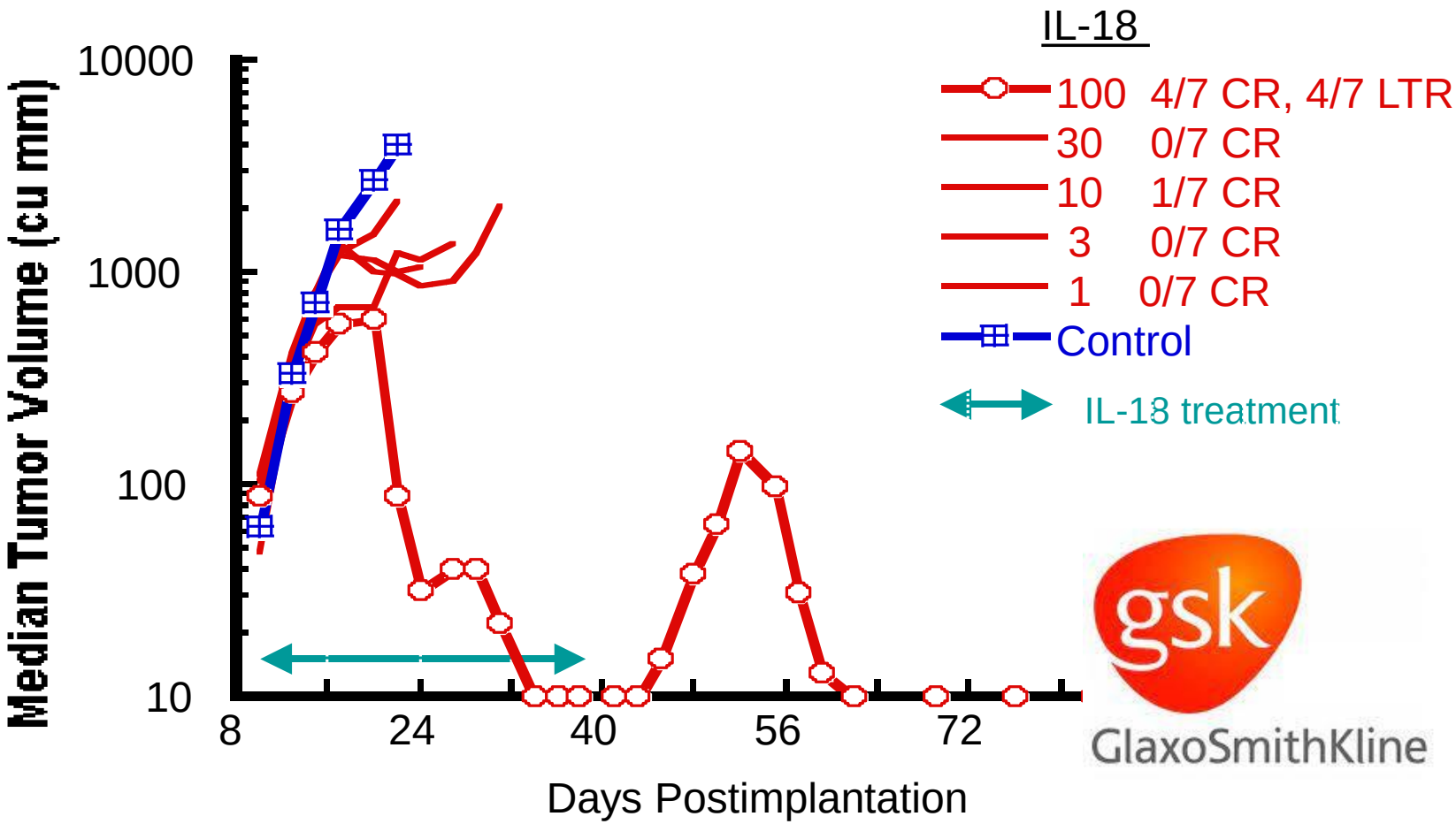
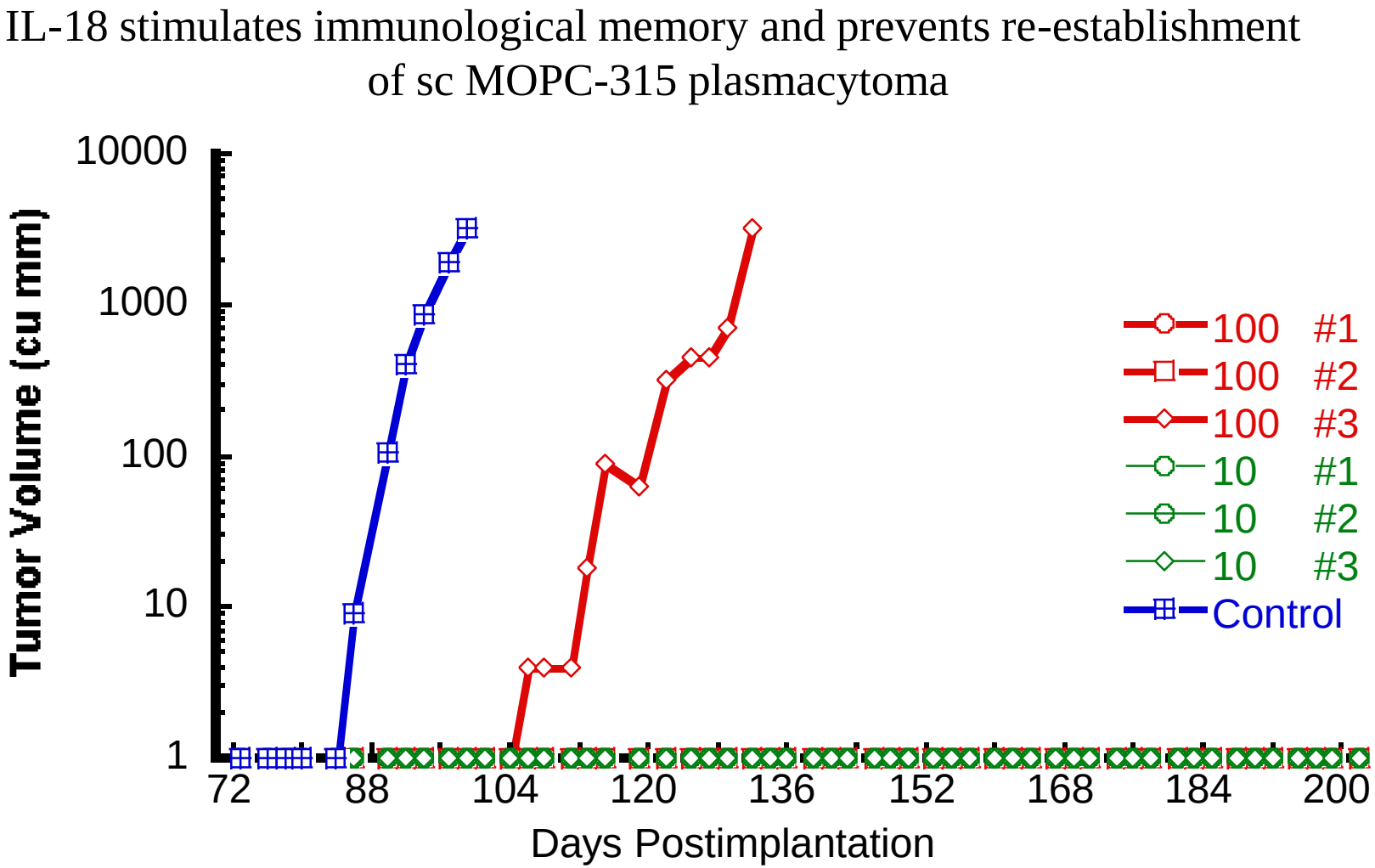
Jonak ZL, Trulli S, Maier C, McCabe FL, Kirkpatrick R, Johanson K, Ho YS, Elefante L, Chen YJ, Herzyk D, Lotze MT, Johnson RK. High-dose recombinant interleukin-18 induces an effective Th1 immune response to murine MOPC-315 plasmacytoma. J Immunother. 2002 Mar-Apr;25 Suppl 1:S20-7. doi: 10.1097/00002371-200203001-00004. PMID: 12048347.



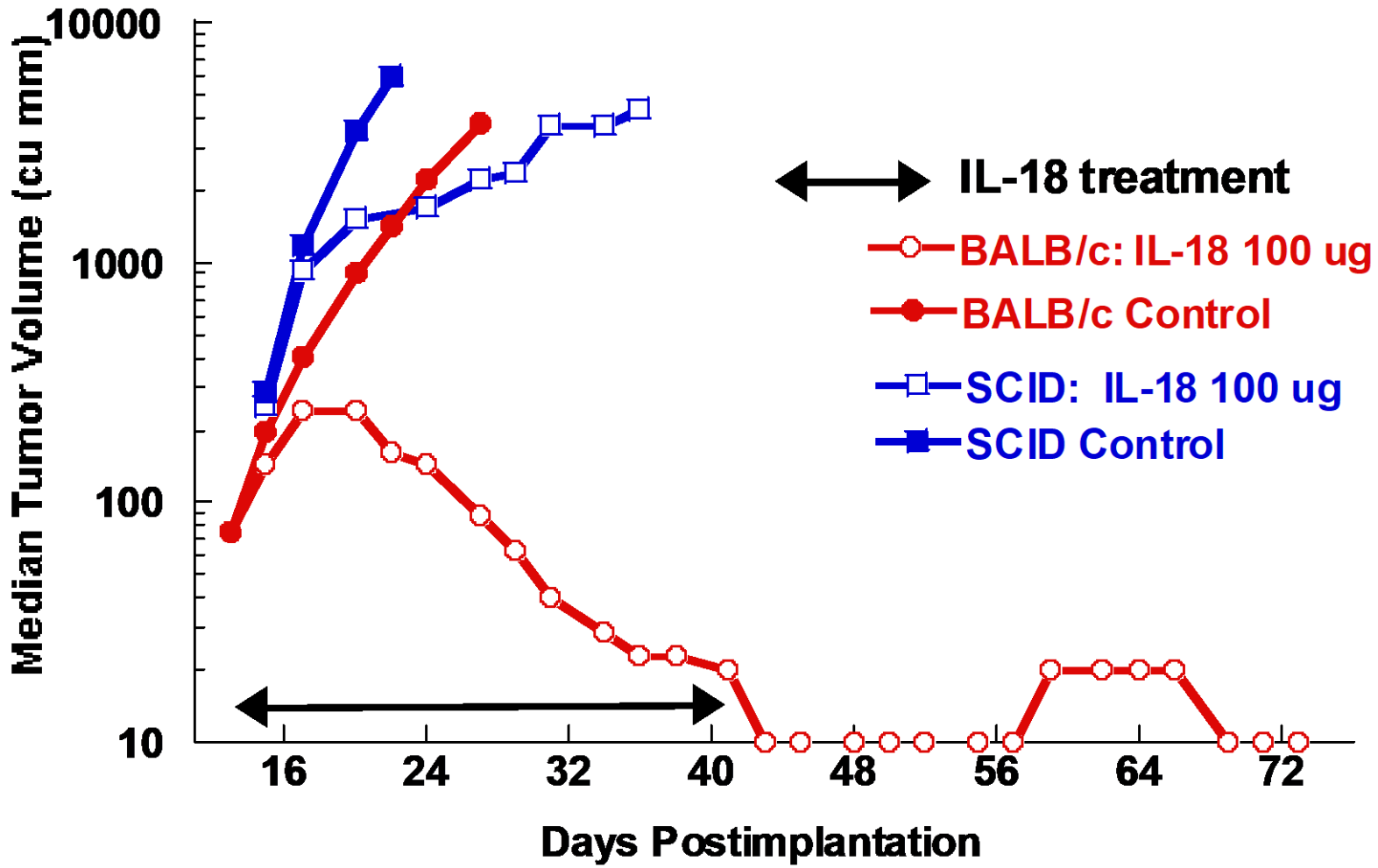
IL-18 in Combination With Chemotherapy

Tumor model	*Chemotherapeutic agent*	*Results*
B16F10 melanoma	cyclophosphamide suboptimal dose	IL-18 enhanced efficacy (tumor growth delay and survival)
Lewis lung carcinoma	cyclophosphamide	At MTD + IL-18 prolonged lifespan (43-53%)
	etoposide	At the highest dose showed increase in lifespan/tumor growth inhibition
Madison 109 lung carcinoma	paclitaxel	No efficacy in combination (IL-18 alone prolonged lifespan by 77% no tumor growth delay)
Mammary adenocarcinoma 16/c	doxorubicin	IL-18 exacerbated toxicity

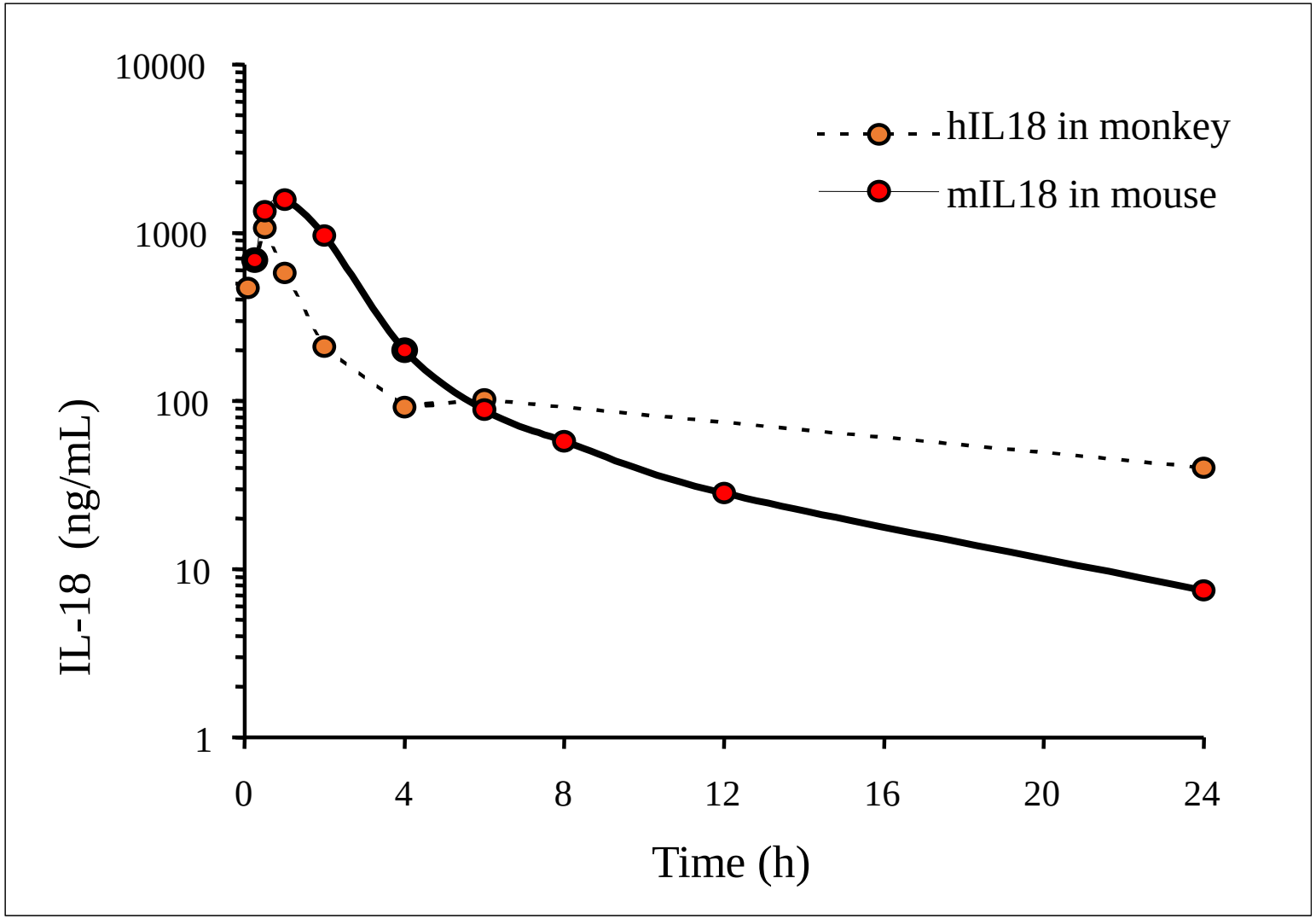
Immunological memory is induced in mice treated with IL-18



IL-18 induced regression of MOPC-315 plasmacytoma in immunocompetent BALB/c, but not in SCID mice



Similar PK Profile of Murine IL-18 in Mice and Human IL-18 in Primates (Single SC Dose of 10 Mg/kg)



In vivo Activities of Human IL-18 Cynomolgus Monkey

Data indicate that human IL-18 is pharmacologically active in cynomolgus monkey

→ Lymphocyte count (lymphopenia)

→ IL-1α and TNF upregulation

→ Upregulation of CD56+, CD16+, CD14+ leukocytes

Herzyk DJ, Soos JM, Maier CC, Gore ER, Narayanan PK, Nadwodny KL, Liu S, Jonak ZL, Bugelski PJ. Immunopharmacology of recombinant human interleukin-18 in non-human primates. Cytokine. 2002 Oct 7;20(1):38-48. doi: 10.1006/cyto.2002.1978. PMID: 12441145.

Pharmacokinetics of Human IL-18 in Cynomolgus Monkey

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Summary of results:

- Plasma concentration declined in a bi-phasic manner.
- Terminal half-life is 15-20 hrs (iv or sc)
- Maximum plasma concentration at 0.5 hr after sc (rapidly absorbed)
- Single *vs* 4 daily sc doses: 3- and 6-fold higher Cmax and AUC
- The sc bioavailability estimated 36%

Clinical and Biological Effects of Recombinant Human Interleukin-18 Administered by Intravenous Infusion to Patients with Advanced Cancer

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rhIL-18 dose, μg/kg (*n*)	Day 1 AUC (h × ng/mL)	Day 5 AUC (h × ng/mL)	Accumulation ratio	Accumulation $t_{1/2}$ (h)
3 (3)	214 (122-247)	461 (273-479)	2.24 (1.87-2.24)	30 (24-42)
10 (4)	353 (228-465)	881 (704-1,045)	2.27 (1.85-4.57)	31 (19-94)
30 (3)	771 (490-858)	2,099 (2,076-2,250)	2.69 (2.62-4.29)	37 (36-38)
100 (5)	627 (574-852)	1,993 (1,774-2,790)	3.27 (2.49-3.70)	41 (27-61)
200 (3)	869 (884-904)	2,252 (1,627-3,108)	2.55 (1.87-3.44)	36 (31-37)
300 (3)	1,983 (1,255-2,392)	5,527 (2,715-6,357)	2.31 (2.22-3.21)	35 (30-44)
600 (3)	3,443 (3,379-3,869)	6,192 (5,393-6,456)	1.60 (1.57-1.91)	26 (25-30)
1,000 (3)	5,941 (5,941-8,523)	9,945 (9,308-17,362)	1.67 (1.57-2.04)	36 (34-36)

Abbreviation: AUC, area under the plasma concentration versus time curve.

Biological effects of rhIL-18 included transient lymphopenia and increased expression of activation antigens on lymphocytes and monocytes. Increases in serum concentrations of IFN-γ, granulocyte macrophage colony-stimulating factor, IL-18 binding protein, and soluble Fas ligand were observed. Two patients experienced unconfirmed partial responses after rhIL-18treatment.

Clin Cancer Res 2006;12(14) July 15, 2006

Contributors

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Medical School
Shigekazu Nagata

Hayashibara Biochemical Labs. Inc.
Masashi Kurimoto

SmithKline Beecham, Inc.
Zdenka Jonak
Randall Johnson
Yen Sen Ho
Frank McCabe
Curtis Maier
Ruth Tal-Singer

An Intimate Cancer Immunotherapy Clinical Trial Protocol Development Program

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- Isabella C. Glitza, MD, PhD – *The University of Texas MD Anderson Cancer Center*
- Michael Lotze, MD, FACS – *Nurix Therapeutics*
- Chris Takimoto, MD, PhD – *IGM Biosciences*