Adeno-Associated Virus for immuno-gene therapy

Paul L. Hermonat, PhD
Professor of Internal Medicine
Professor of OB/GYN
VA Research Career Scientist
Mehta-Stebbins Chair in Medicine
Director, Gene Therapy Program

Immuno-gene therapy against cancer.

1) Carl June, U Pennsylvania
2) Paul Fisher, Columbia U, NY, NY
3) Walter Storkus, U Pittsburgh
4) Zdenek Hel, U Alabama-Birmingham
5) Gianpietro Dotti, Johns Hopkins, Baltimore
6) David Baltimore, Calif Inst Tech
7) Marco Davila, Sloan Kettering Inst

Virus for gene therapy

Retroviruses (Retrov) (8kb, RNA, enveloped) pathogenic

MMLV based- *activates proto-oncogene by insertional mutagenesis, contaminating wild type retroviruses, express fast- then decline*

ADVERSE REACTIONS: ***causes cancer (lymphoma) humans and monkeys***

transgene inactivation in vivo common

easily inactivated, must often incubate target cells with producer cells for high transduction

NEW HOPE- Lentivirus vectors based on HIV, appear to have better expression capabilities, BUT are they safe?

Adenoviruses (Ad) (35kb, DNA, non-enveloped) pathogenic

Episomal, transient, express fast, express stron, then decline

highly immunogenic causing inflammation and anaphylactic shock

ADVERSE REACTIONS: ***has caused the death of a patient in Philadelphia anaphylactic shock***

Adeno-Associated Virus (AAV) (5kb, DNA, non-enveloped) non-pathogenic,

chromosomal integration, not strongly immunogenic, no disease

long term expression in vivo, 3.7 years is record thus far

tough, stores well

NO ADVERSE REACTIONS: ***…of a patient in Targeted Genetics trial, due to systemic histoplasmosis-bad patient management, appears not due to AAV itself, one report of tumors in rodents.***

Immuno-gene therapy against cancer.

Our research has covered:

1) Cervical Cancer: HPV E6 and E7
2) Prostate Cancer: PSA and PSMA
3) Breast Cancer: BA46
4) Multiple Myeloma: HM1.24
5) HCV: multiple antigens
6) HBV: multiple antigens

TREATMENT OF CANCER
What new treatments can be developed?

- **STANDARD TREATMENT**

Surgery

Radiotherapy

Chemotherapy

50% still die

- **EXPERIMENTAL TREATMENT**

Immunotherapy

Gene therapy

First surgery, then Chemotherapy Immunotherapy Radiotherapy

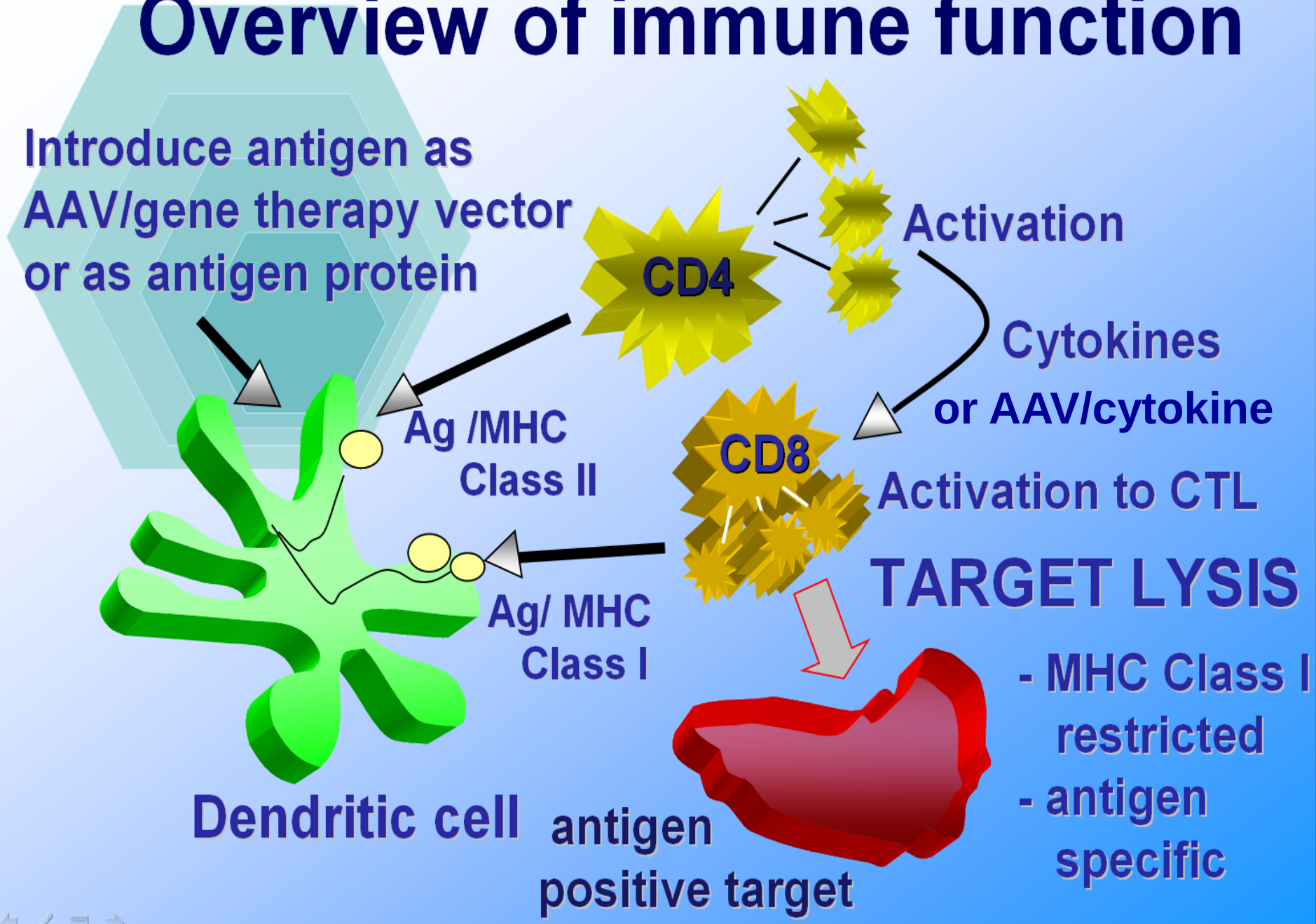
- Often effective but Toxic
- Dose-limitation
- Non–specific: kills non-cancer cells also
- Active killing only during treatment

- Specific
- Less Toxic
- No dose limitation
- Very limited side effects
- Effective on a long- term basis

Myths about AAV as an Immuno-therapeutic vector

1) Myth AAV does not transduce immune cells, eg. macrophages, dendritic cells, T cells, etc.
2) Myth AAV-DC delivery cannot stimulate imm response,
3) Myth is that Ad is
4) Myth AAV does not chromosomally integrate into primary human immune cells

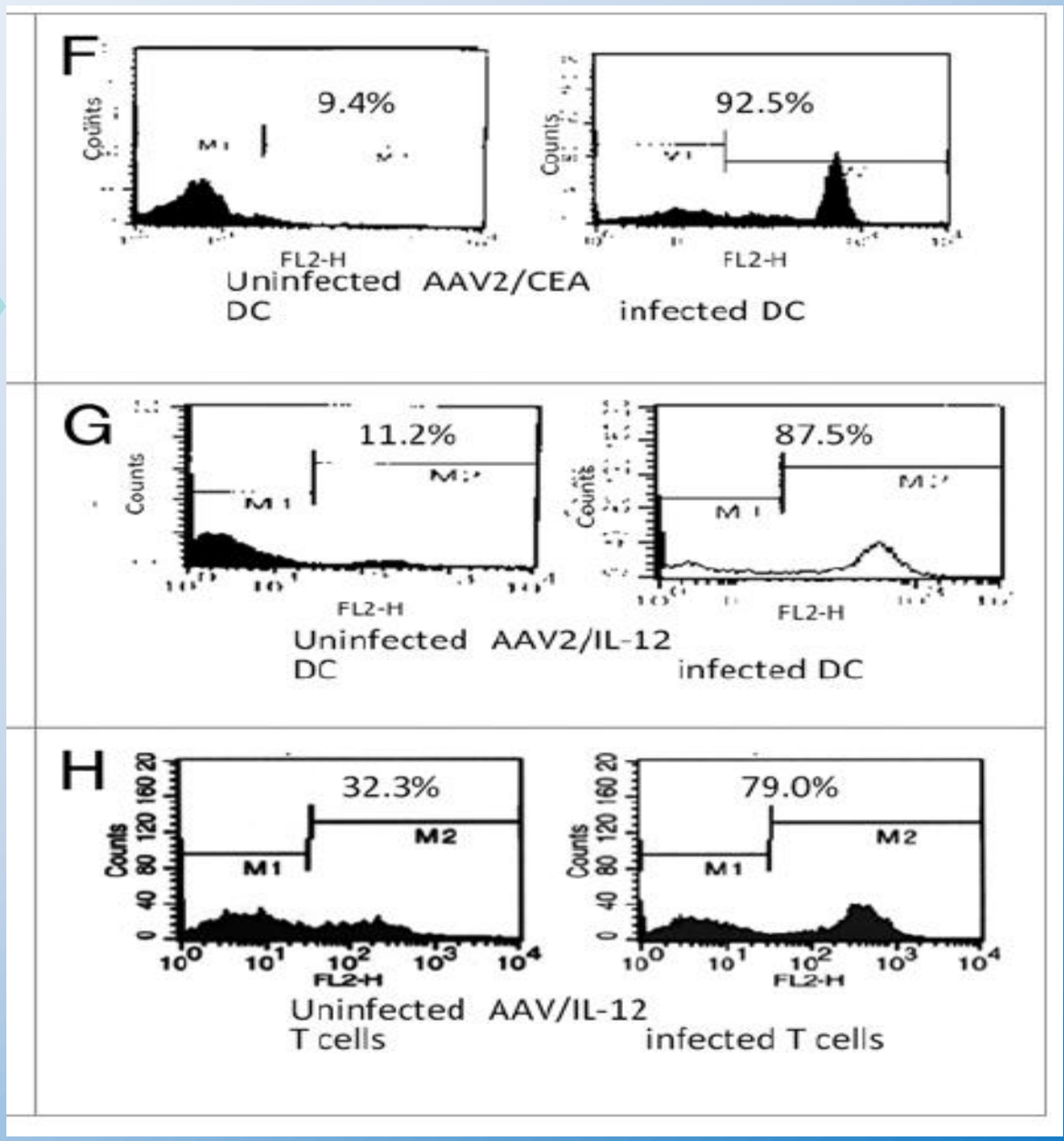
Overview of immune function



Myths

AAV2 transduces ~90% of dendritic cells

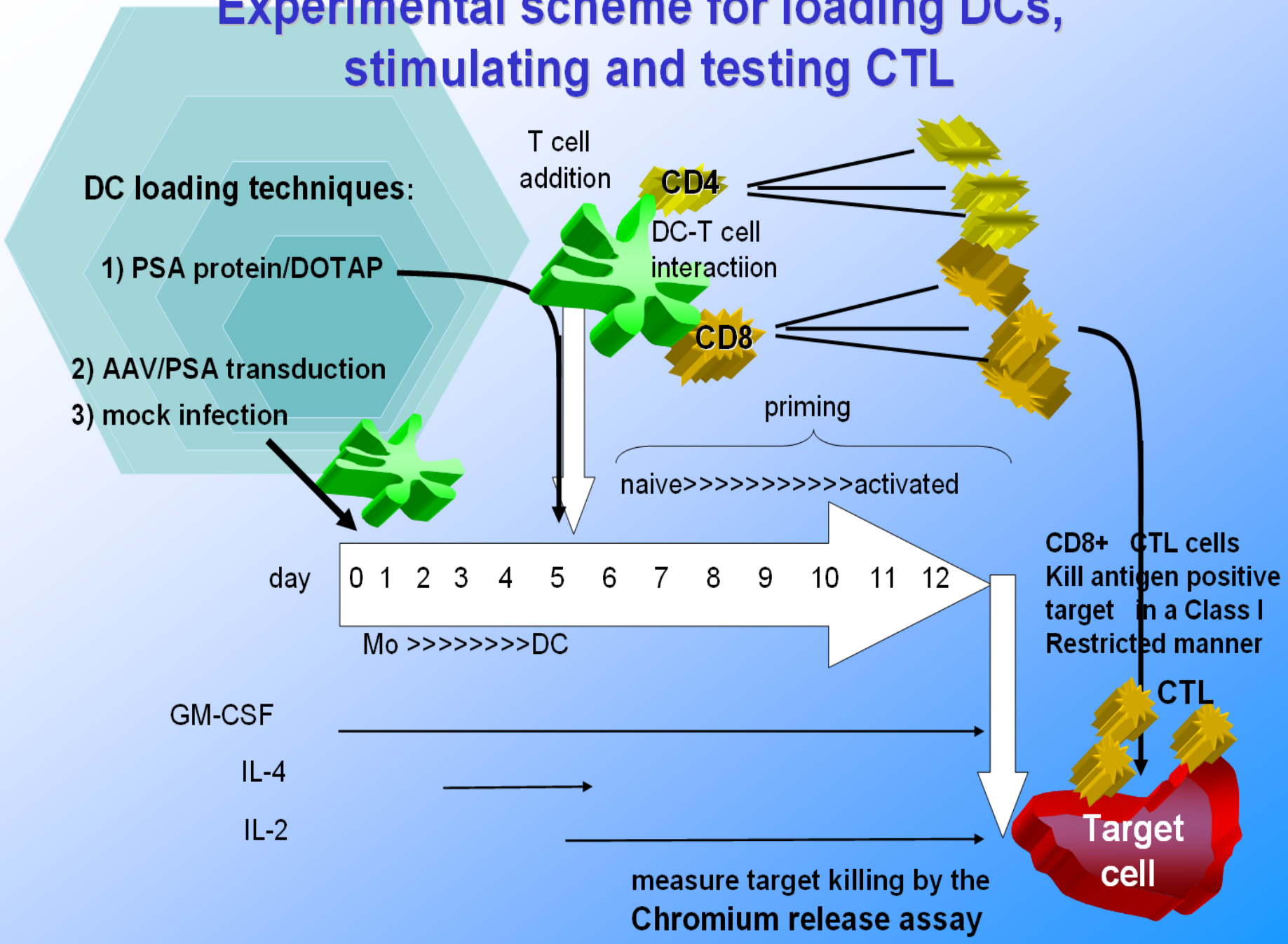
AAV2 transduces ~80% of T cells



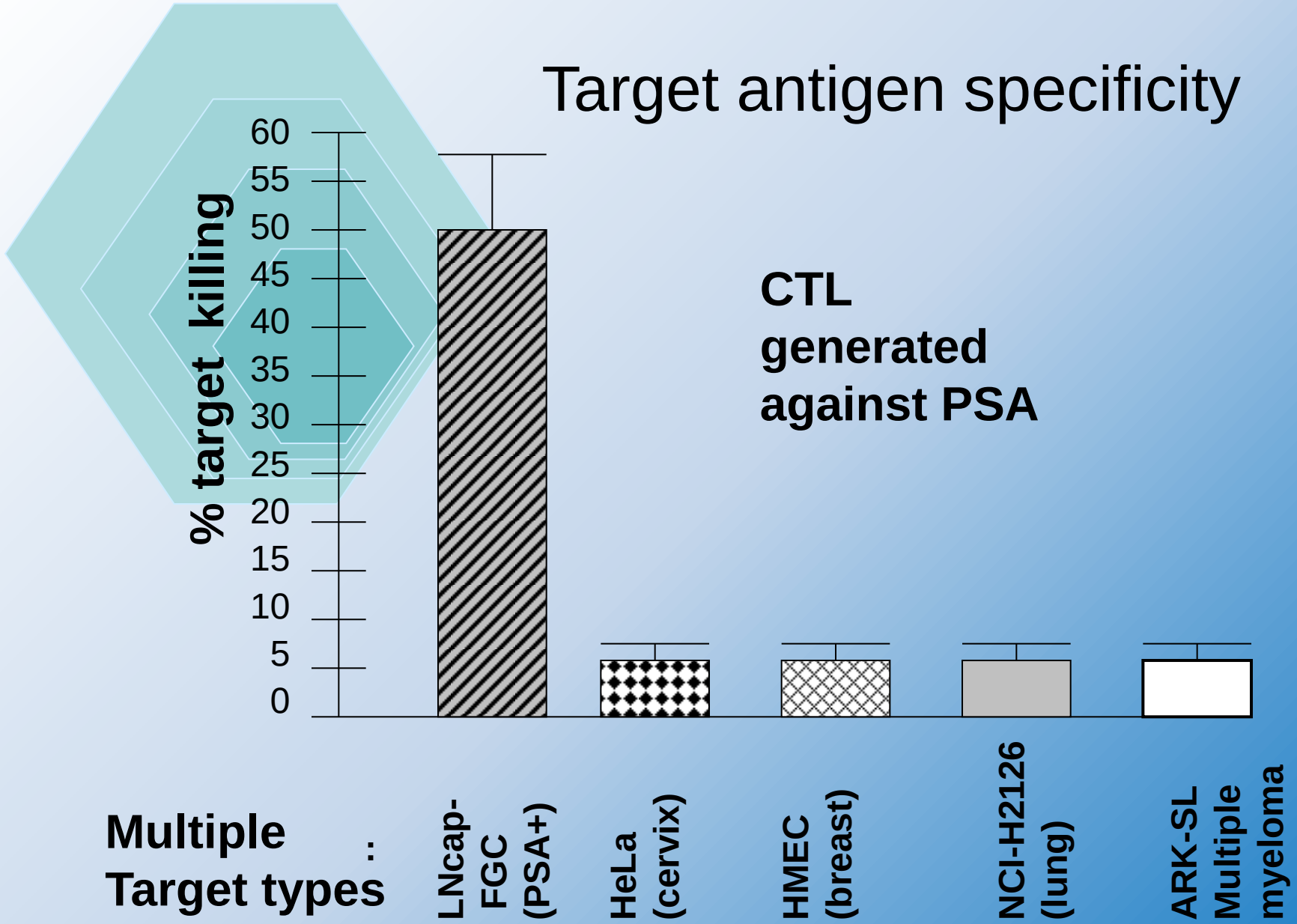
Myths about AAV as an Immuno-therapeutic vector

1) Myth AAV does not transduce immune cells, eg. macrophages, dendritic cells, T cells, etc.
2) Myth AAV-DC delivery cannot stimulate imm response,
3) Myth is that Ad is better than AAV at generating CTL
4) Myth AAV does not chromosomally integrate into primary human immune cells

Experimental scheme for loading DCs, stimulating and testing CTL



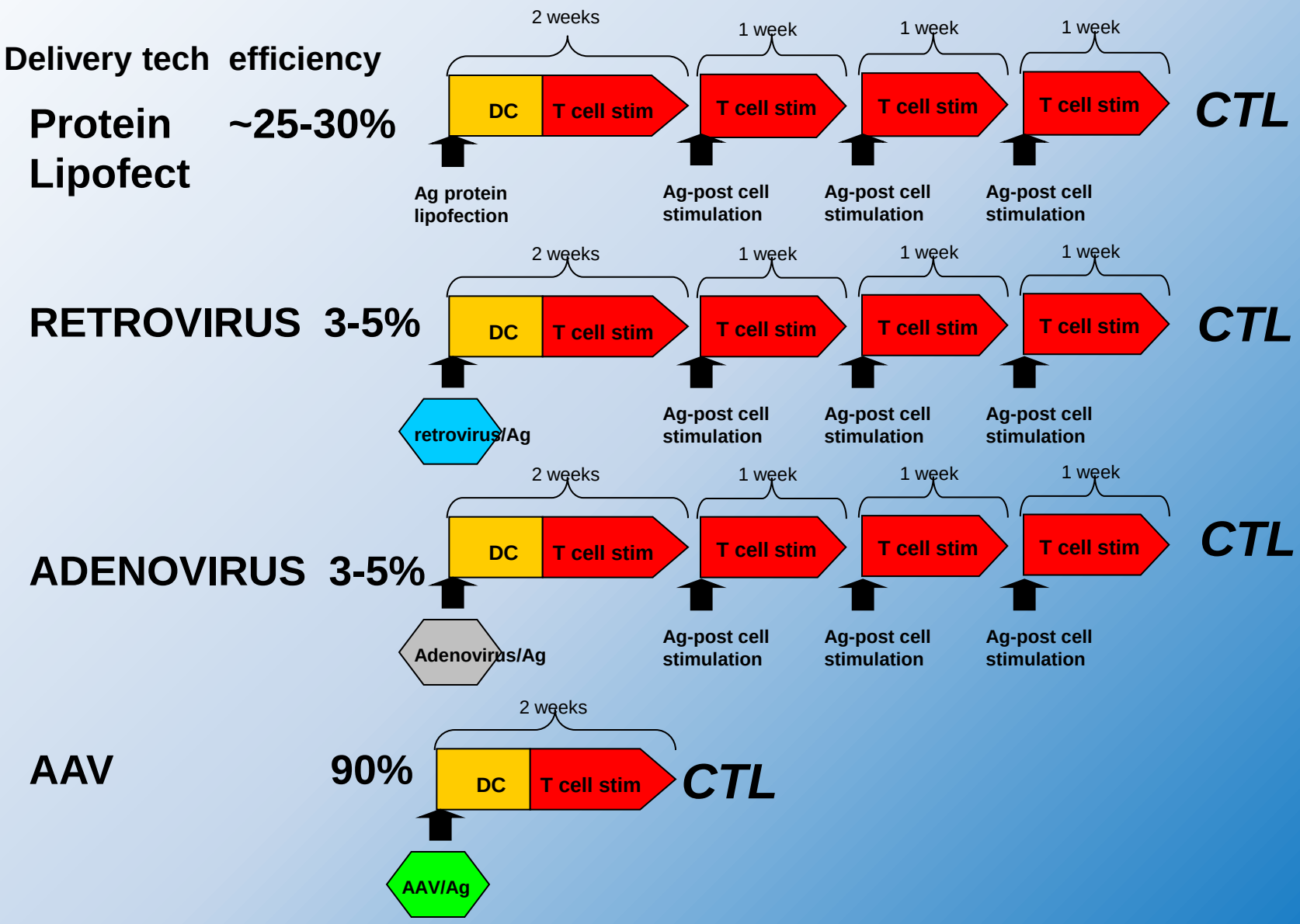
Target antigen specificity



Myths about AAV as an Immuno-therapeutic vector

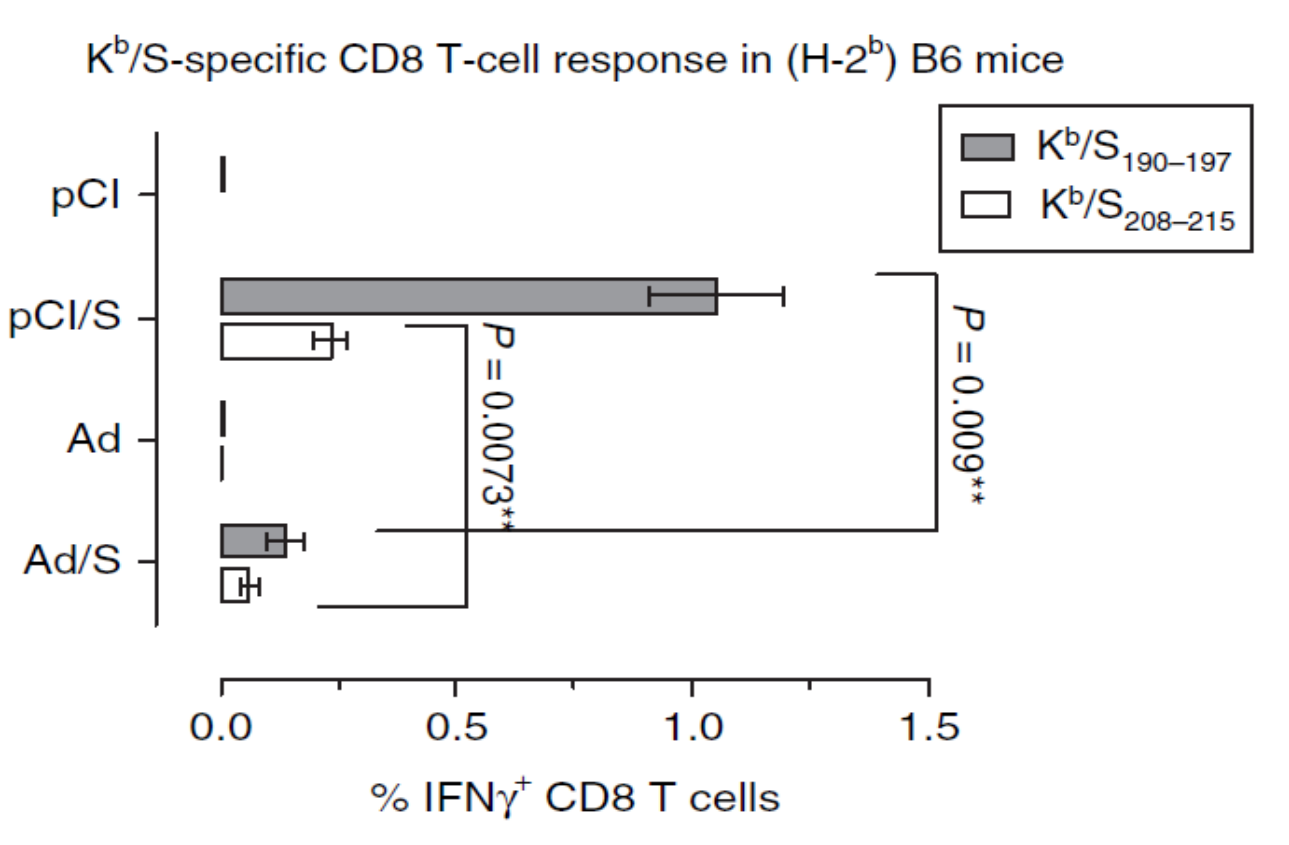
1) Myth AAV does not transduce immune cells, eg. macrophages, dendritic cells, T cells, etc.
2) Myth AAV can not stimulate CTL response,
3) Myth is that Ad is better at CTL stimulation than AAV
4) Myth AAV does not chromosomally integrate into primary human immune cells

Generation of CTL by loading DC: A comparison of speed of various techniques



Use of *Adenovirus vector* as vaccine acts as CTL "sink" and weakens CTL response against the delivered antigen

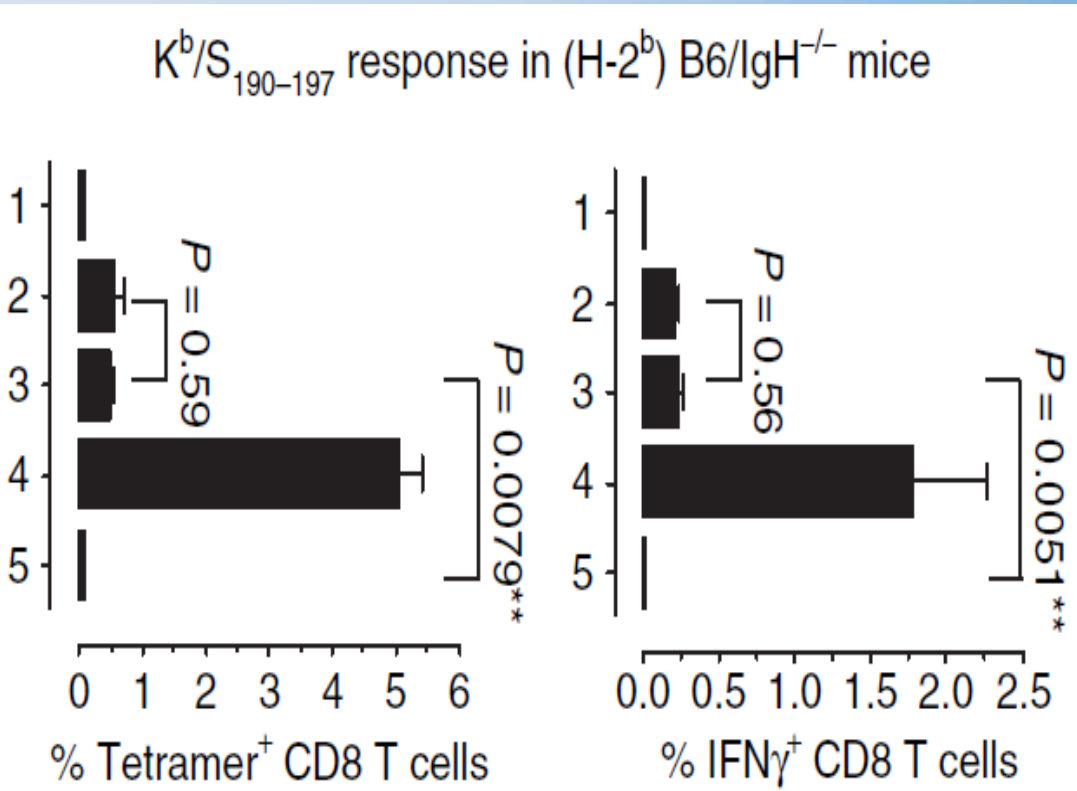
Schirmbeck R, Reimann J, Kochanek S, Kreppel F. The Immunogenicity of Adenovirus Vectors Limits the Multispecificity of CD8 T-cell Responses to Vector-encoded Transgenic Antigens. Molec Ther 16: 1609-1616.



Use of *Adenovirus vector* as vaccine acts as CTL "sink" and weakens CTL response against the delivered antigen

Schirmbeck R, Reimann J, Kochanek S, Kreppel F. The Immunogenicity of Adenovirus Vectors Limits the Multispecificity of CD8 T-cell Responses to Vector-encoded Transgenic Antigens. Molec Ther 16: 1609-1616.

Group	Prime	Boost
1	--	--
2	Ad/S	--
3	Ad/S	Ad/S
4	Ad/S	pCI/S
5	Ad	Ad/S

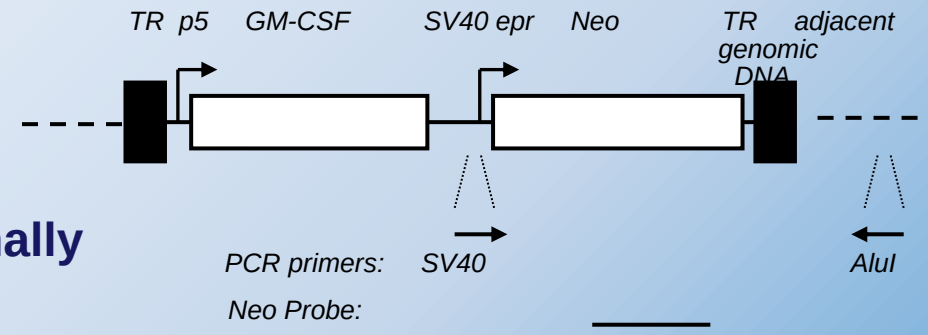


Myths about AAV as an Immuno-therapeutic vector

1) Myth AAV does not transduce immune cells, eg. macrophages, dendritic cells, T cells, etc.
2) Myth AAV can not stimulate CTL response,
3) Myth is that Ad is better at CTL stimulation than AAV
4) Myth AAV does not chromosomally integrate into primary human immune cells

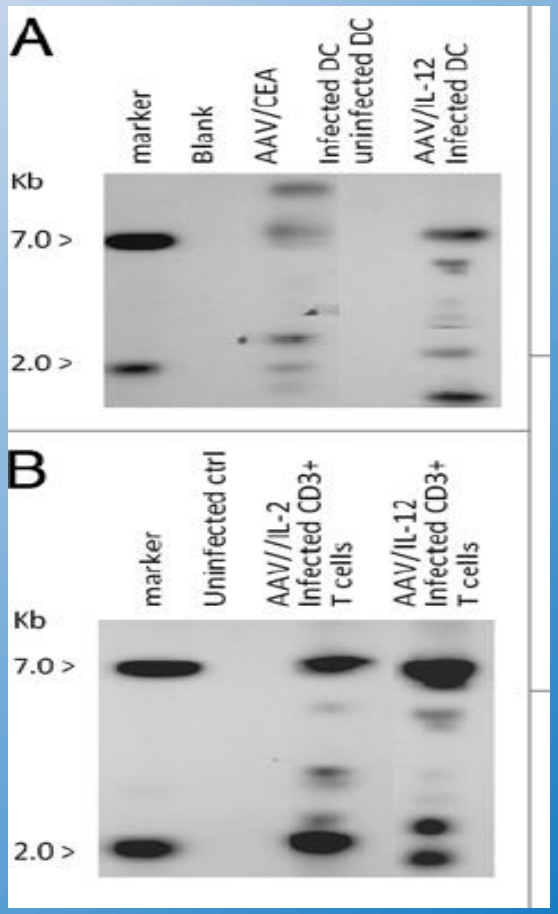
Myths

Assay for identifying chromosomally Integrated AAV provirus



Chromosomal integration into DC

Chromosomal integration into T cells



Killing by CD8+ CTL

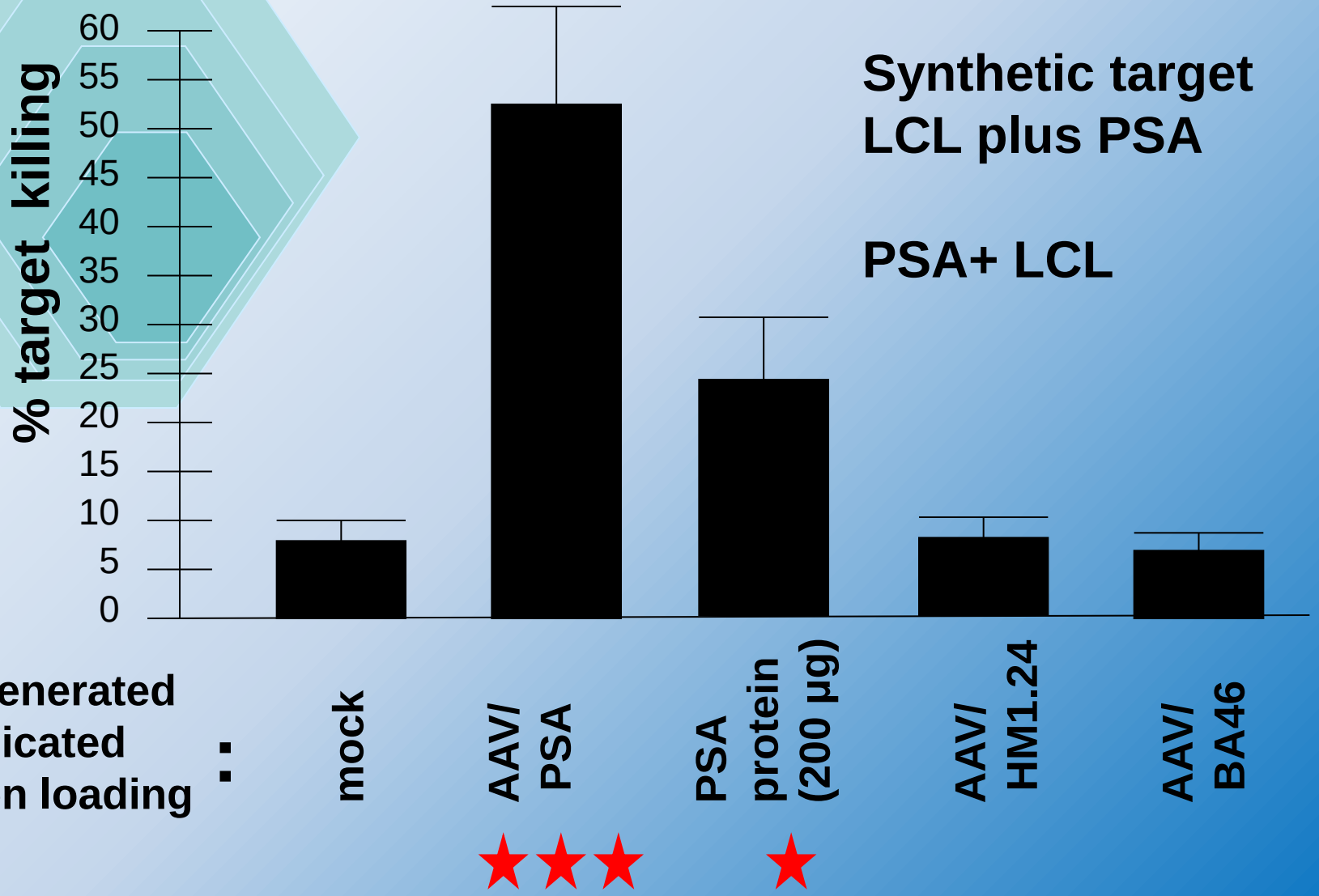
1) CD8+ CTL killing should be antigen-specific

2) CD8+ CTL killing should be HLA Class I restricted

3) CD8+ CTL killing should be loading-dose dependent

4) CD8+ CTL killing should be CTL number/dose dependent

DC antigen loading specificity



Killing by CD8+ CTL

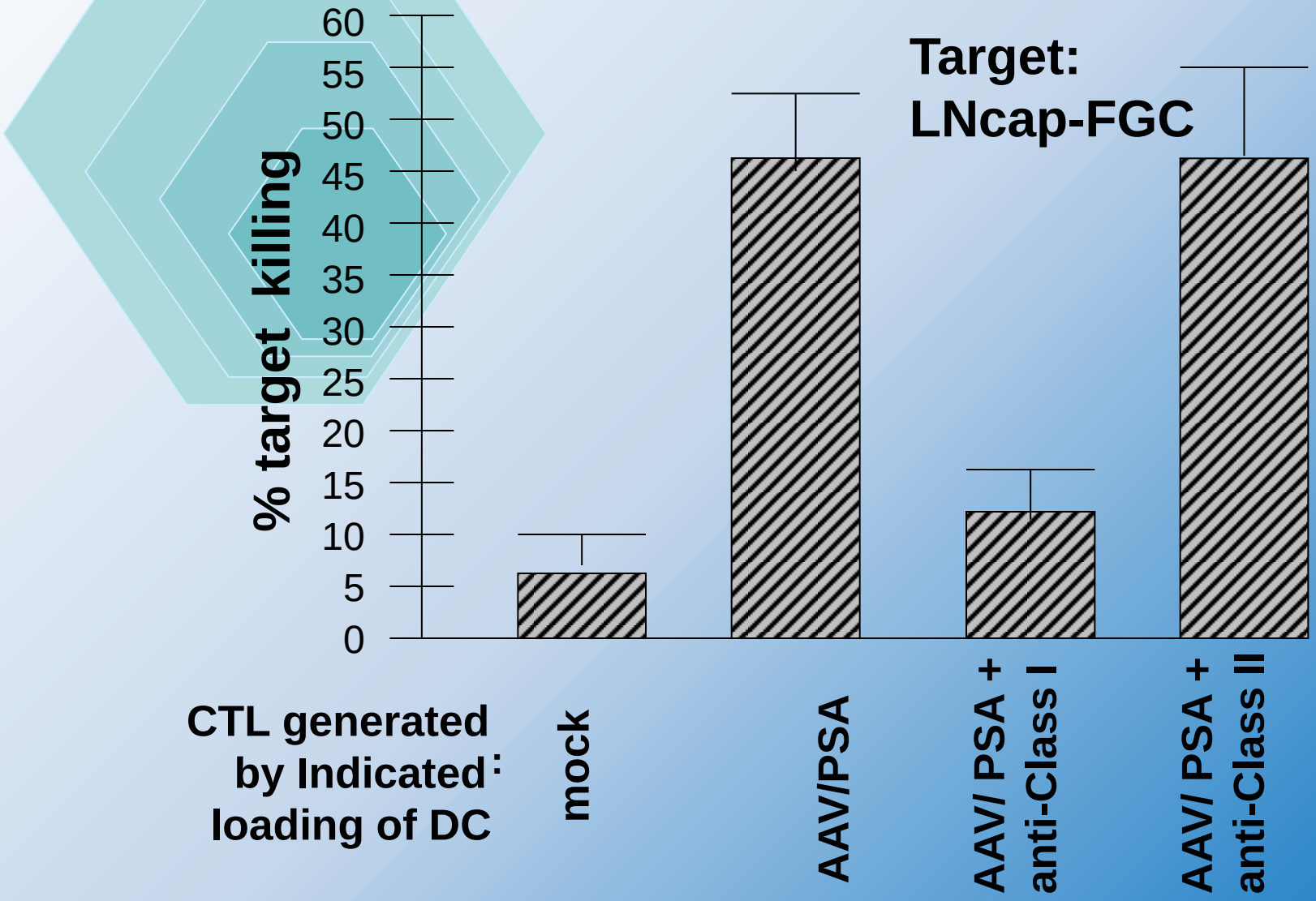
1) CD8+ CTL killing should be antigen-specific

2) CD8+ CTL killing should be HLA Class I restricted

3) CD8+ CTL killing should be loading-dose dependent

4) CD8+ CTL killing should be CTL number/dose dependent

Killing of prostate cancer cell line is HLA/MHC Class I restricted



Killing by CD8+ CTL

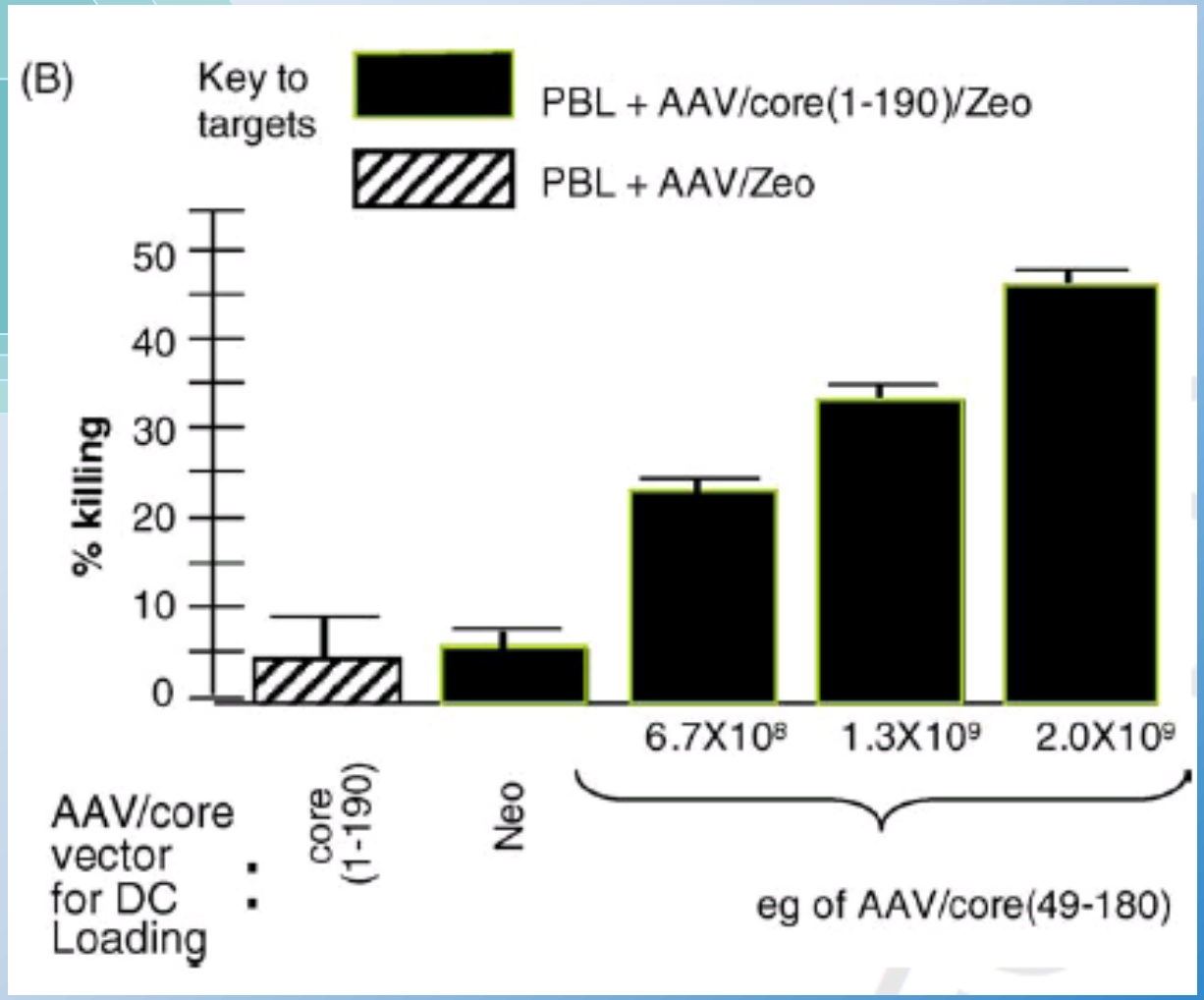
1) CD8+ CTL killing should be antigen-specific

2) CD8+ CTL killing should be HLA Class I restricted

3) CD8+ CTL killing should be loading-dose dependent

4) CD8+ CTL killing should be CTL number/dose dependent

The higher the amount of AAV/core antigen virus the dendritic cells are infected with the higher the stimulation of HCV core-specific CTL killers.



Killing by CD8+ CTL

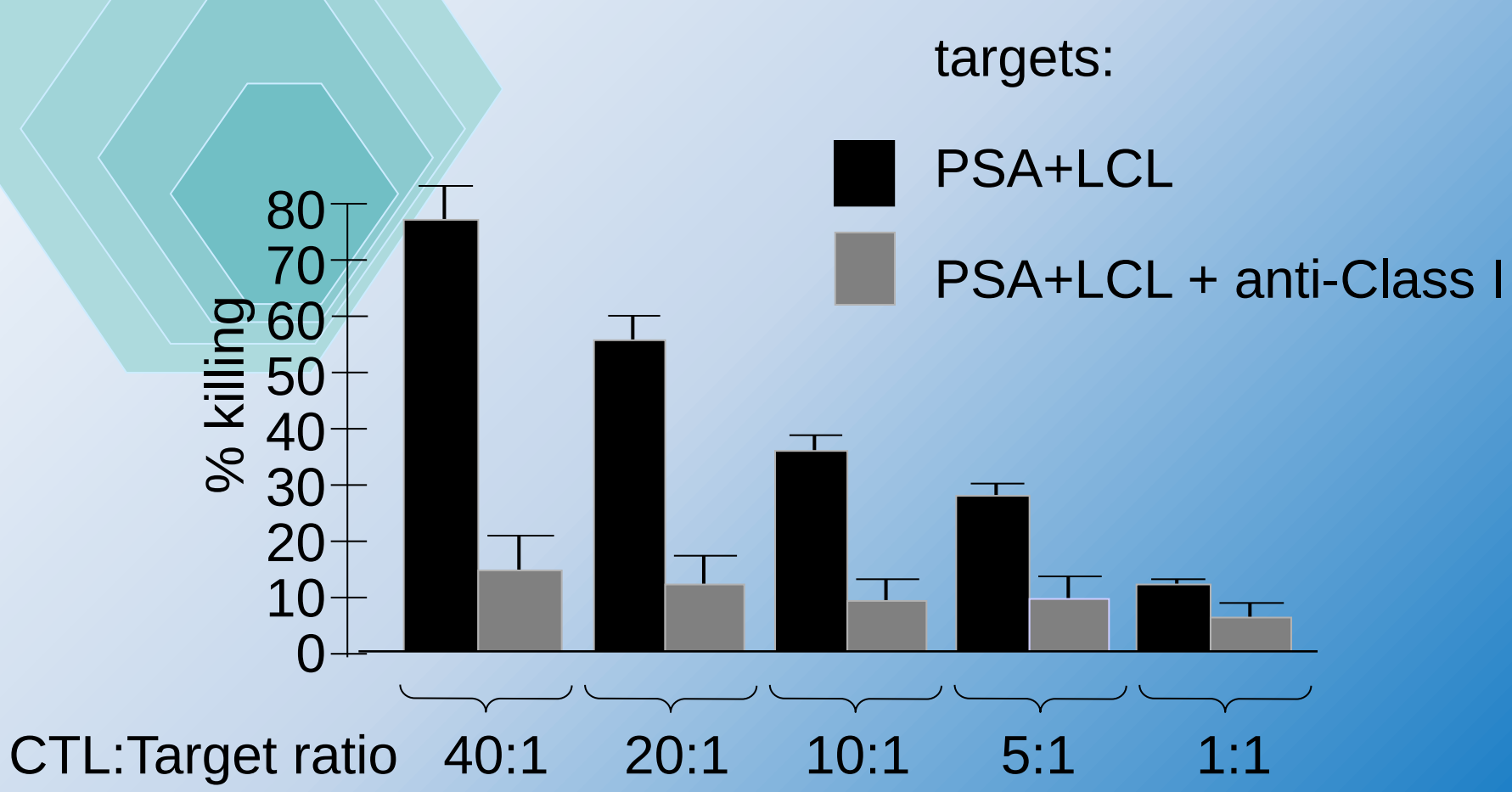
1) CD8+ CTL killing should be antigen-specific

2) CD8+ CTL killing should be HLA Class I restricted

3) CD8+ CTL killing should be loading-dose dependent

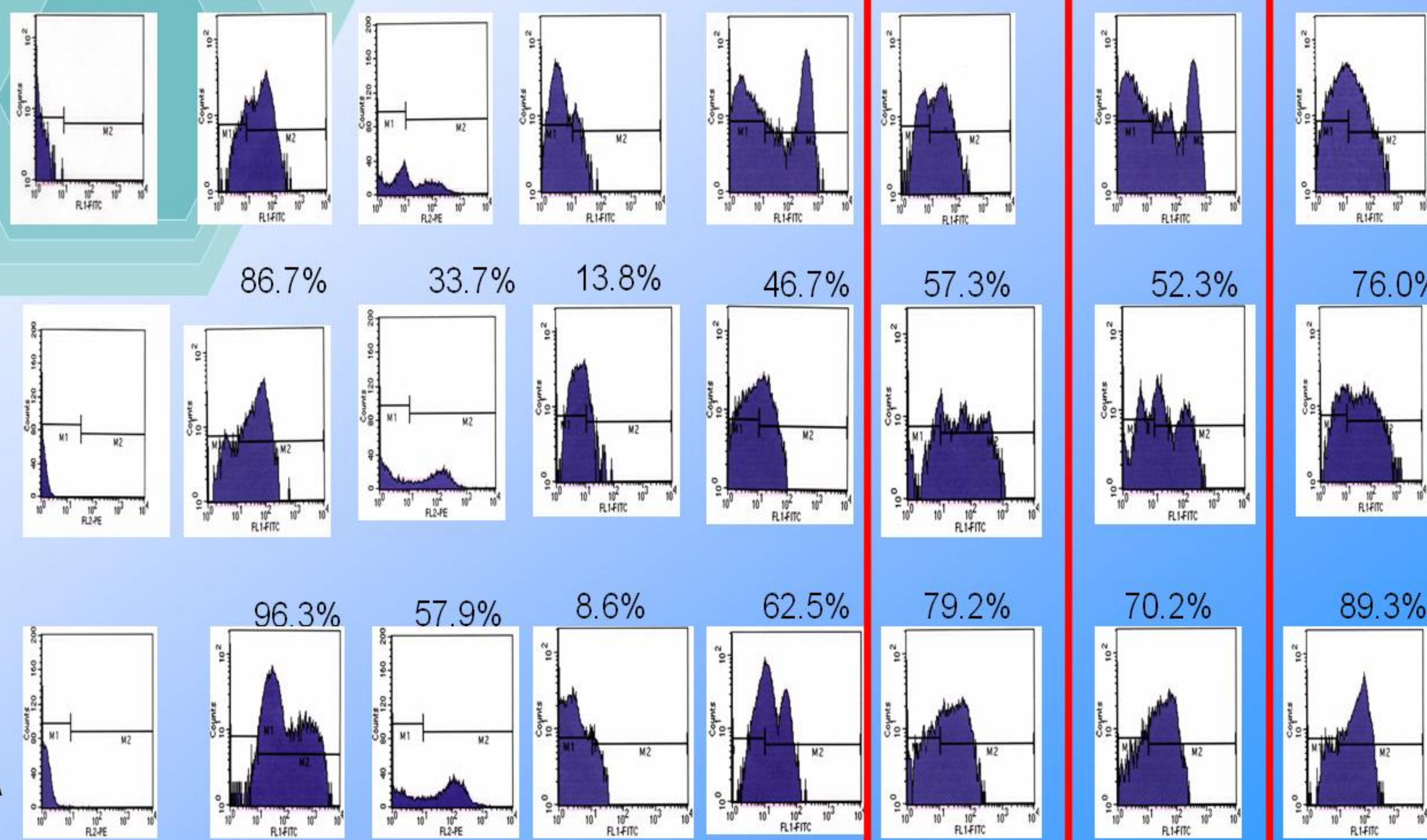
4) CD8+ CTL killing should be CTL number-dose dependent

Killing is CTL dose dependent



DC Markers

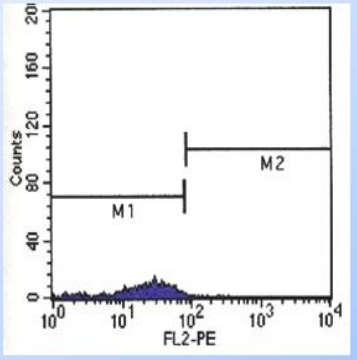
	Isotype	HLA-DR	CD1a	CD14	CD40	CD80 B7-1	CD83	CD86 B7-2
normal DC		83.2%	32.2%	11.9%	48.0%	61.7%	48.3%	72.3%
DC+ mock		86.7%	33.7%	13.8%	46.7%	57.3%	52.3%	76.0%
DC + AAV/PSA		96.3%	57.9%	8.6%	62.5%	79.2%	70.2%	89.3%



The Level of CD25 in T Cell Populations

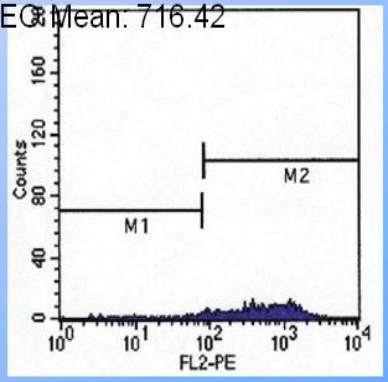
*PSA protein: 200 ug/ml AAV/PSA: 1000ul

Isotype GEO Mean: 68.98



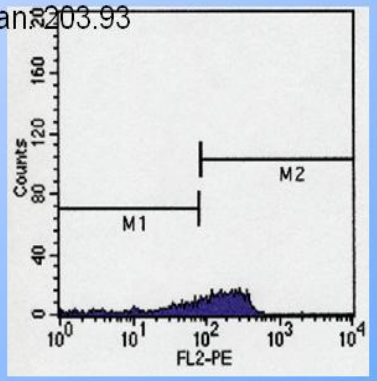
Lysate/mock: 63.71%

GEO Mean: 716.42



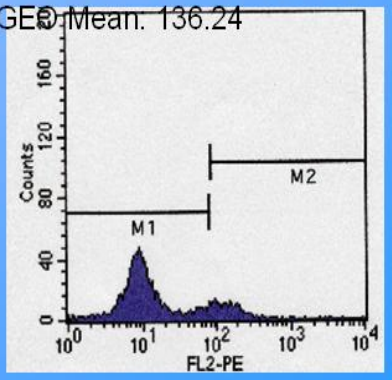
PSA protein 49.85%

GEO Mean: 203.93



AAV/PSA 14.86%

GEO Mean: 136.24



Conclusions

1) rAAV/Ag transduce DC at high frequency
2) These DC are powerful, stimulate CTL in one week
3) CD8+ CTL killing is antigen-specific
4) CD8+ CTL killing is MHC Class I restricted
5) CD8+ CTL killing is loading-dose dependent
6) CD8+ CTL killing is CTL dose dependent
7) AAV-loaded DC overexpress CD80
8) CD8+/CD4+ ratio is high
9) CD8+/CD56+ ratio is high
10) T cell CD69+ levels are high
11) CD25 levels are low

Attempts to improve CD8+ CTL killing by cytokine gene delivery

Th1 response/ CD8+ CTL enhancing cytokines:

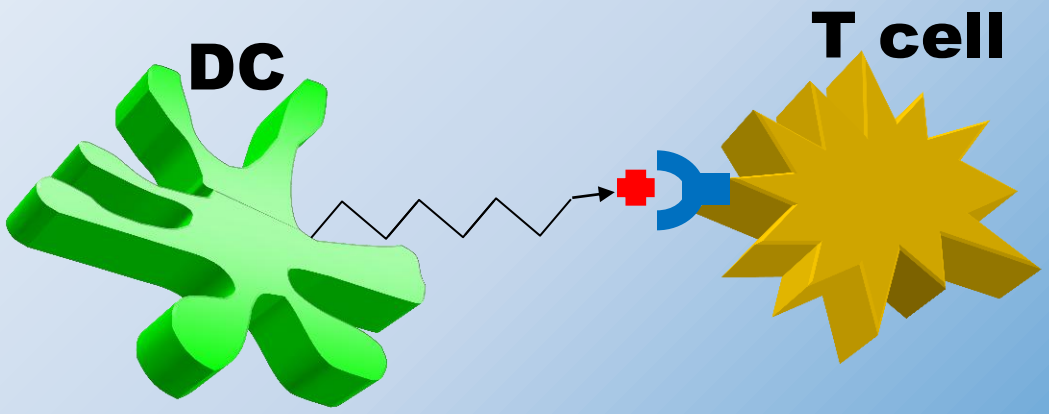
1) IL-7
2) IFN-gamma
3) IL-12
4) IL-15
5) IL-18
6) IL-21
7) Etc., etc., etc

Improving CD8+ CTL killing by cytokine gene delivery

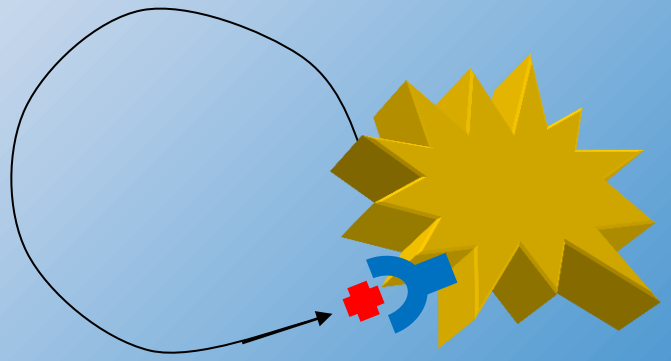
1) What is the true action of cytokines?
2) What arethe immune cell types are affected?
3) What is the optimal immune cell type for expressing each Th1 response cytokine?

Modes of actions of cytokines

Paracrine



Autocrine

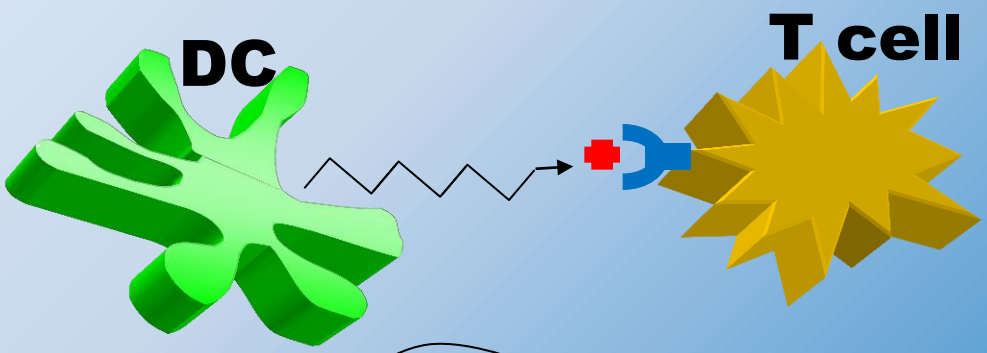


Intracrine

Studies of three chemokines on identifying the optimal mode of action.

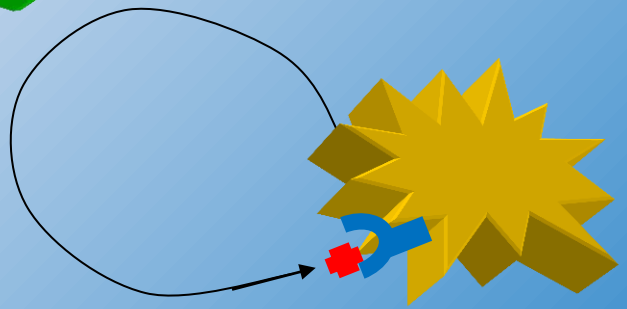
Paracrine

IL-12



Autocrine

IL-7, IFNgamma



Intracrine

IL-12 ?

AAV/IL-12 gene delivery into dendritic cells enhances CTL stimulation, providing evidence for IL-12 intracrine activity.

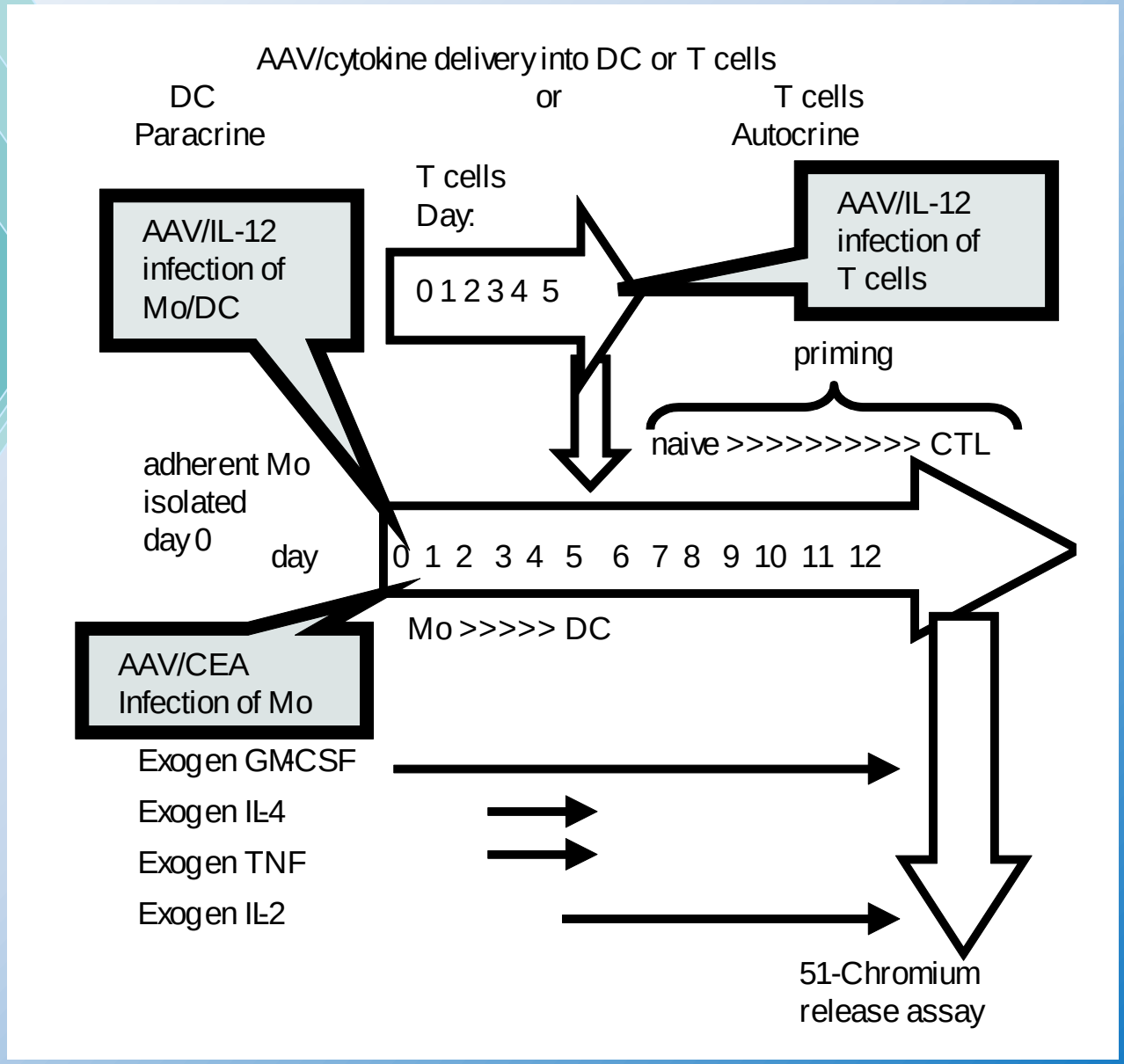
Chang-Xuan You[2,3], Min Shi[1,2,*], Yong Liu[1,3,*], Maohua Cao[1], Rongcheng Luo[2,#], Paul L. Hermonat[1,3]

Department of [1]Internal Medicine, and [3]Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, AR, USA 72205. [2]Department of Oncology, Nanfang Hospital, Southern Medical University, Guangzhou, China.

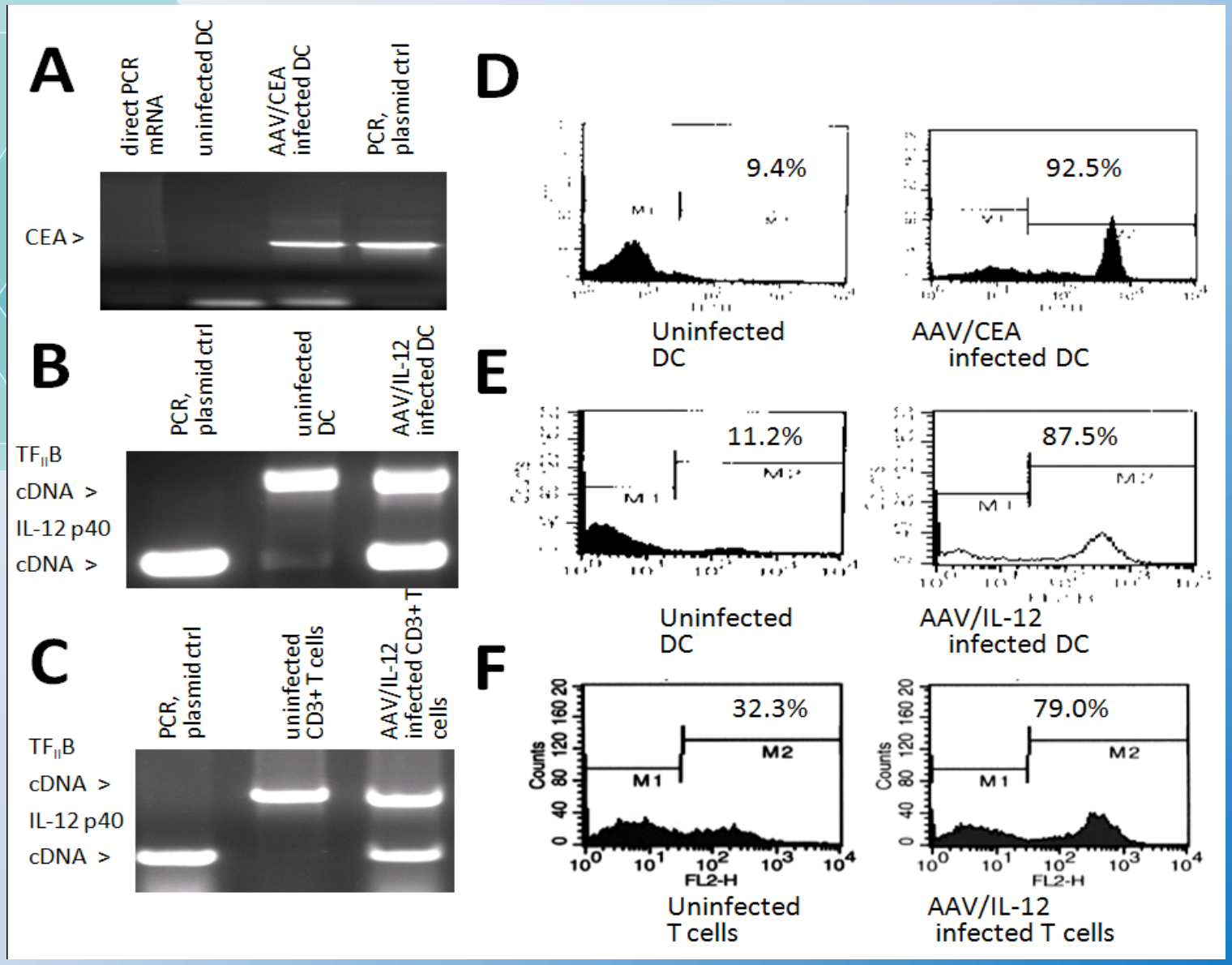
*The first three authors contributed equally to this manuscript.
Dr. Rongcheng Luo is co-contributing author

Abstract:
Adoptive transfer of antigen-specific cytotoxic T lymphocytes (CTL) holds significant promise in treating cancer and Th1 response cytokines are critical for their stimulation. Recently we reported that interleukin 7- (IL-7) and interferon gamma- (IFN-γ) autocrine/T cell gene delivery resulted in superior CTL stimulation over paracrine/DC delivery. In sharp contrast, neither IL-2 autocrine or paracrine gene delivery gave any advantage over exogenous IL-2 addition. IL-12 is yet another important Th1 cytokine which affects both DC and T cells. Here, using adeno-associated virus type 2 (AAV) gene delivery, it was found that IL-12-paracrine/DC gene delivery resulted in significantly superior stimulation of carcinoembryonic antigen (CEA)-specific CTL killing over that induced by autocrine gene delivery (or exogenous IL-12 addition). This difference is surprising as both AAV/IL-12-transduced T cells and DC secreted approximately the same level of IL-12. Paracrine IL-12 gene delivery also resulted in highest IL-12/IL-10 secretion ratio by DC, highest T cell IFN-γ production, highest T cell proliferation, highest CD69+/CD8+ levels, and lowest level of CD25+/CD4+ Treg. These data strongly suggest that the primary activity of IL-12 during CTL generation is upon DC. The high activities of AAV/IL-12-transduced DC are consistent with there being a unique activity for IL-12 within the DC, not involving its surface receptor; an "intracrine" activity. Given the plethora of IL-12 studies, these data also suggest that the gene delivery approach could be useful for uncovering new cytokine activities and mechanism(s) of action gone unrecognized by conventional immunologic assays. Finally, these data further suggest AAV/IL-12 cytokine gene delivery into DC may have utility in immunotherapy protocols involving antigen-specific CTL.

IL-12 gene delivery into T cells or DC. Which is best?

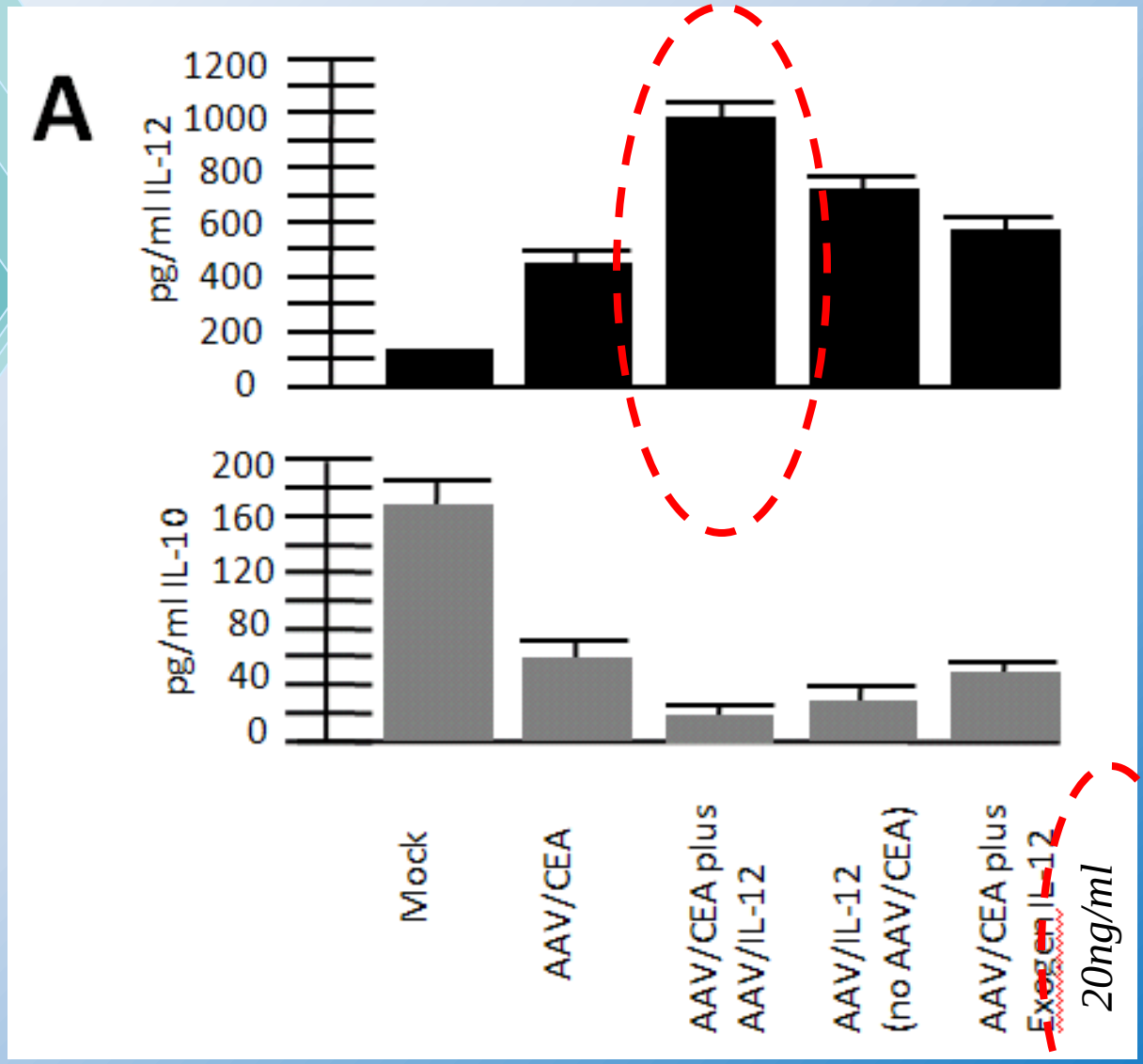


IL-12 gene delivery and expression into DC and T cells.



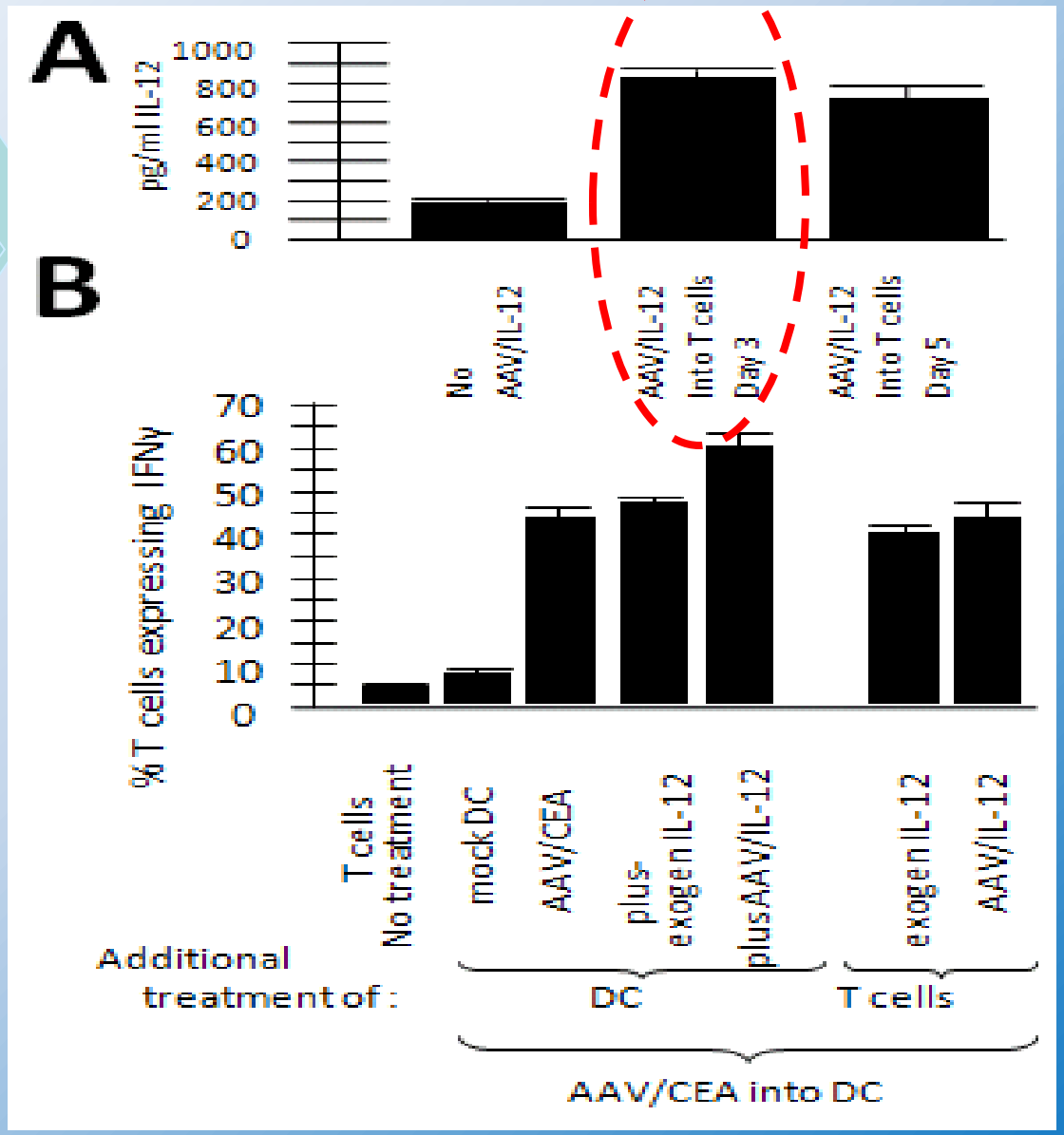
IL-12 gene delivery into DC cells: Secretion and ratio of IL-12/IL-10 expression.

Figure 3 shows AAV/IL12-transduced DC secreted ~950 pg/ml IL12.

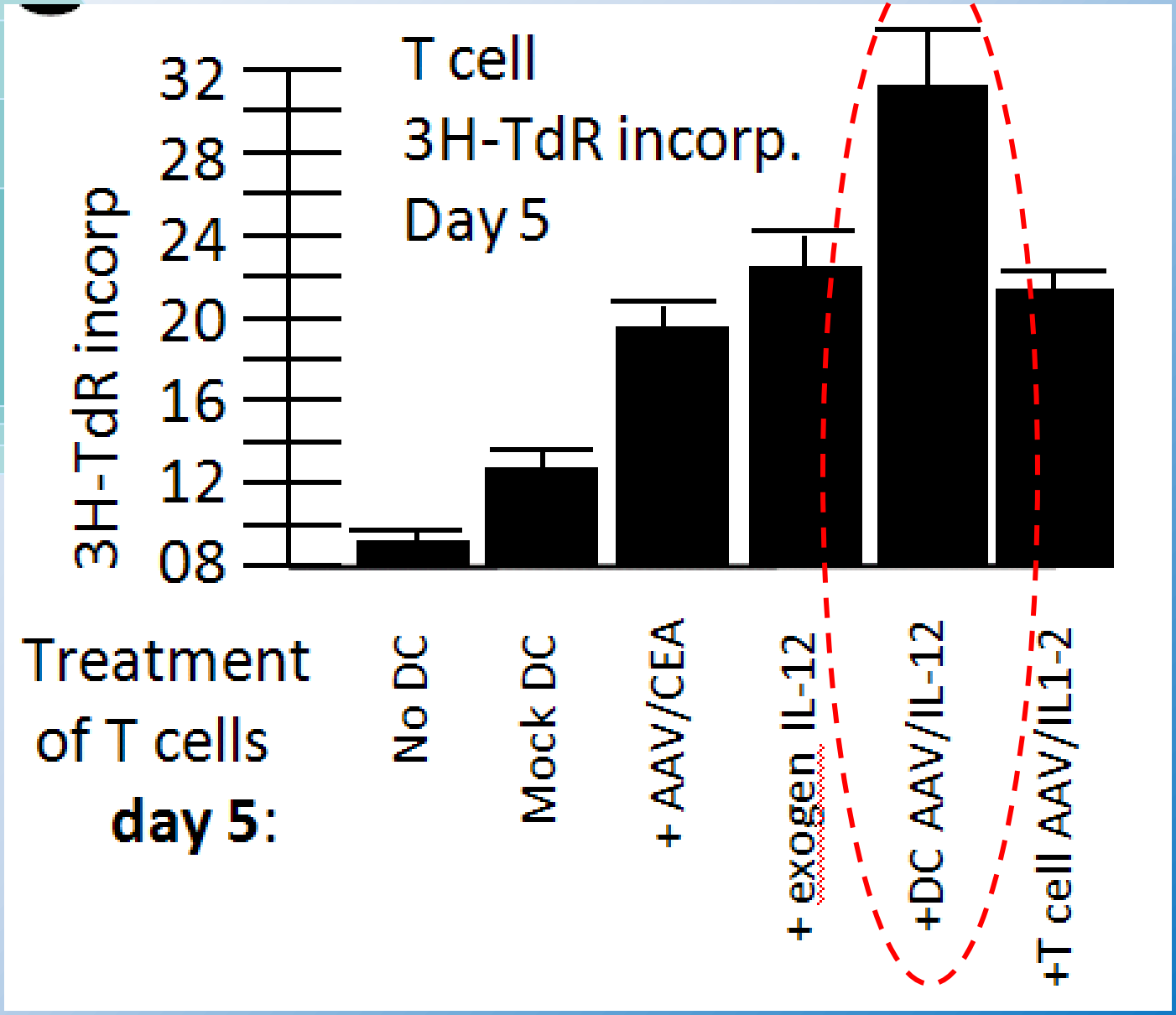


A: Secretion of IL-12 from T cells.
B: IFNgamma expression in T cells.

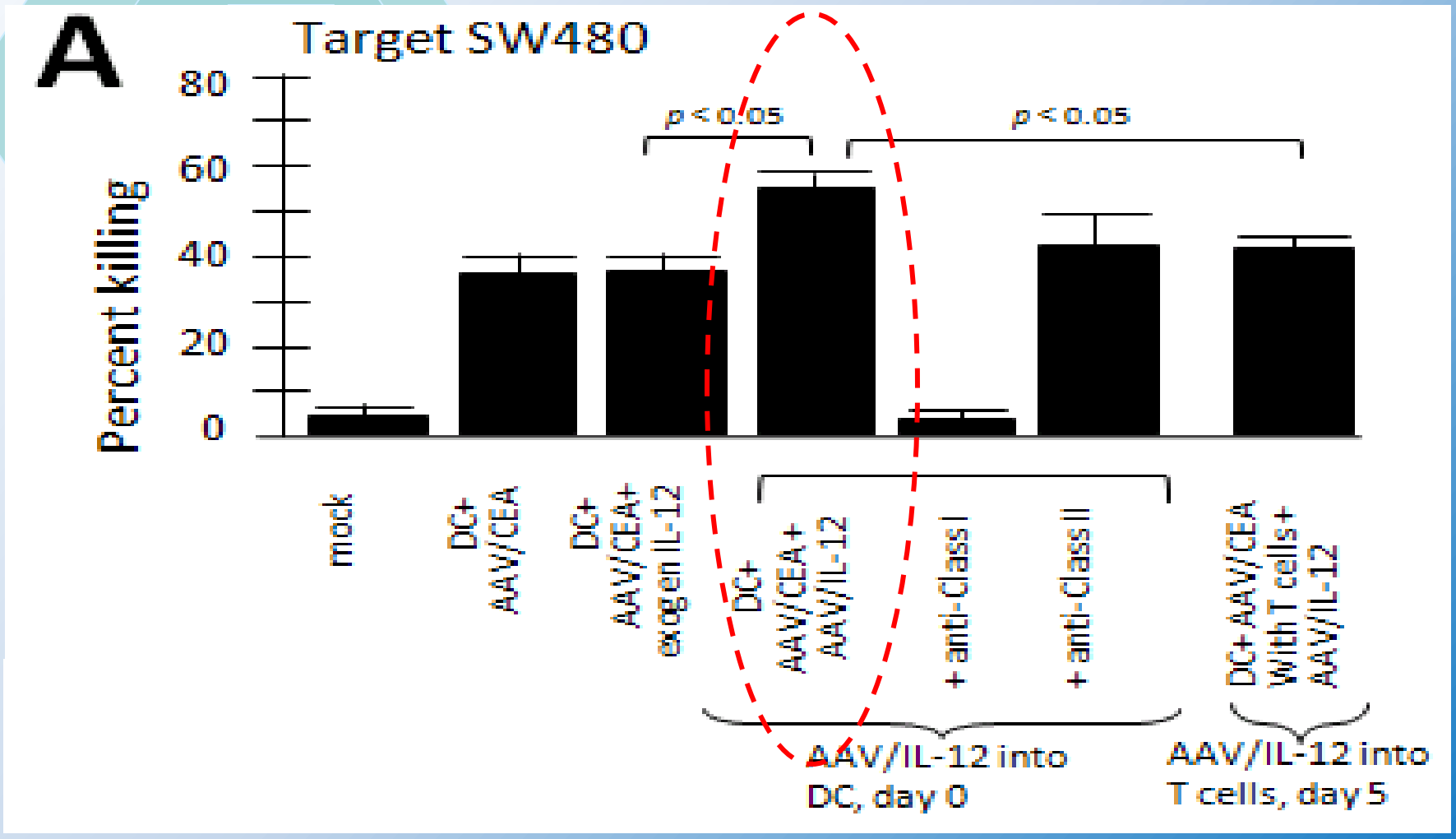
Figure 4 shows AAV/IL12-transduced T cells secreted ~850 pg/ml IL12.



Proliferation of T cells highest with IL-12 Delivery into DC.



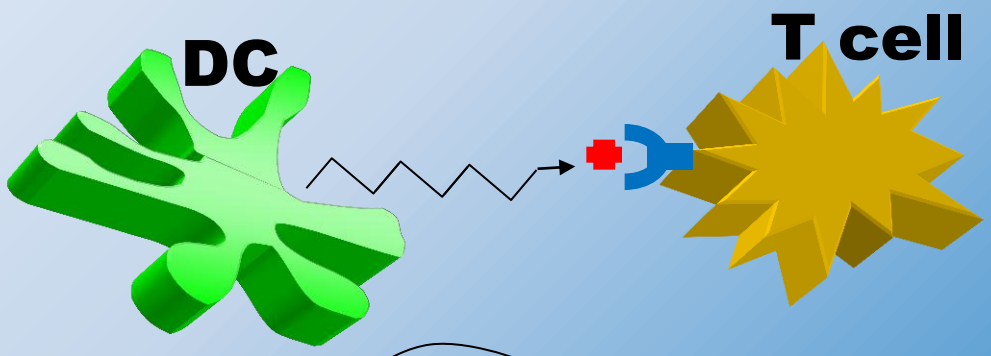
IL-12 gene delivery into DC allows those DC to stimulate *the* best, outstanding CTL killers.



Studies of three chemokines on identifying the optimal mode of action.

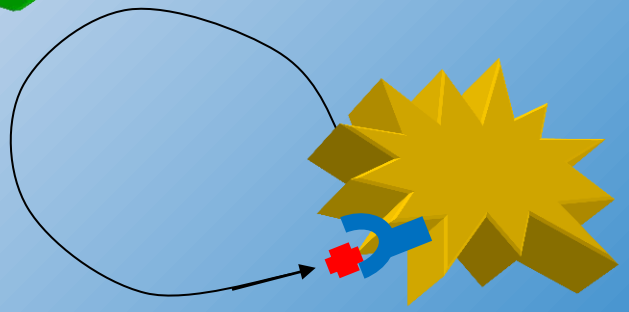
Paracrine

IL-12



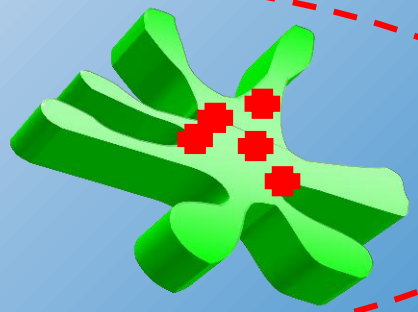
Autocrine

IL-7, IFNgamma

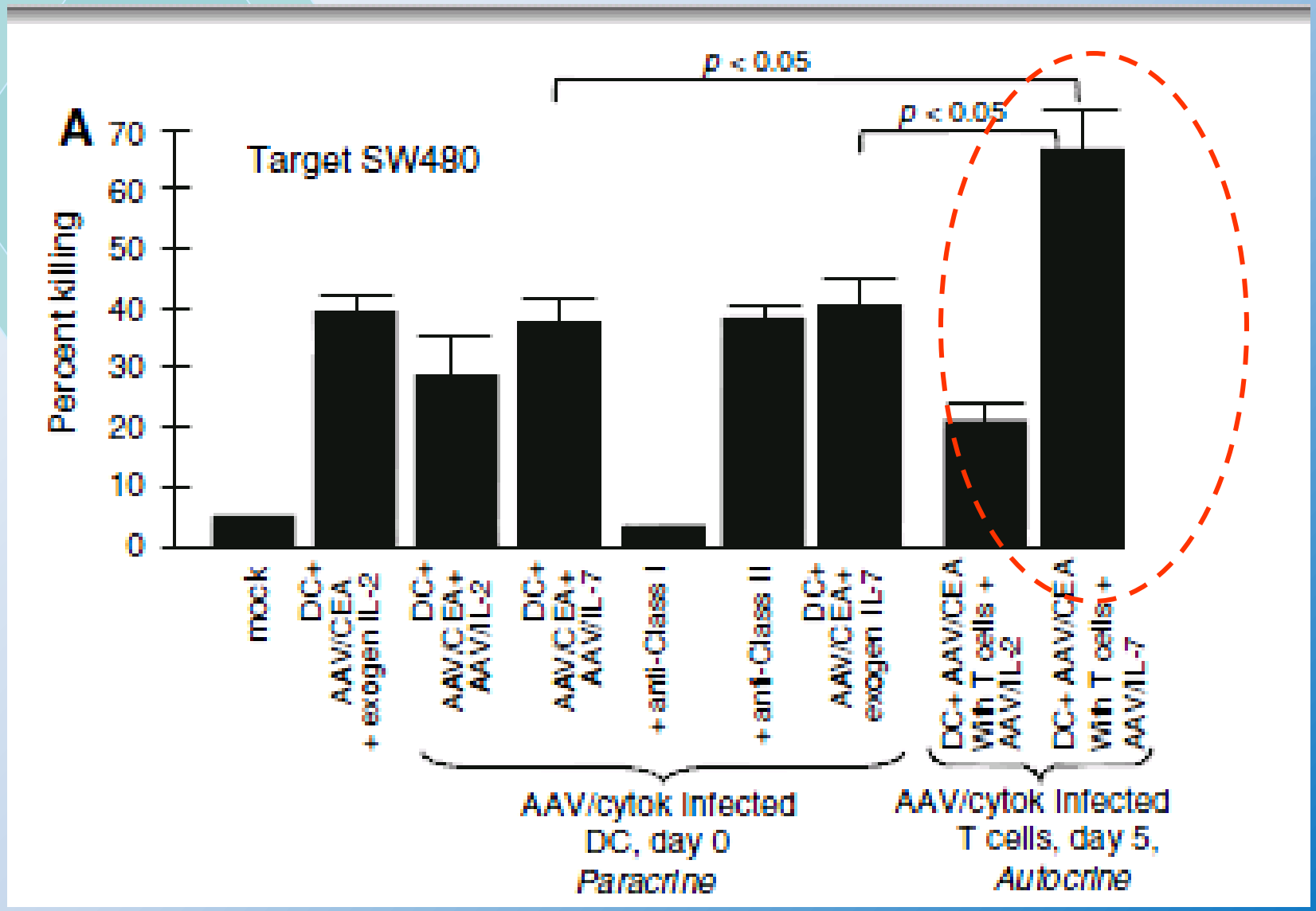


Intracrine

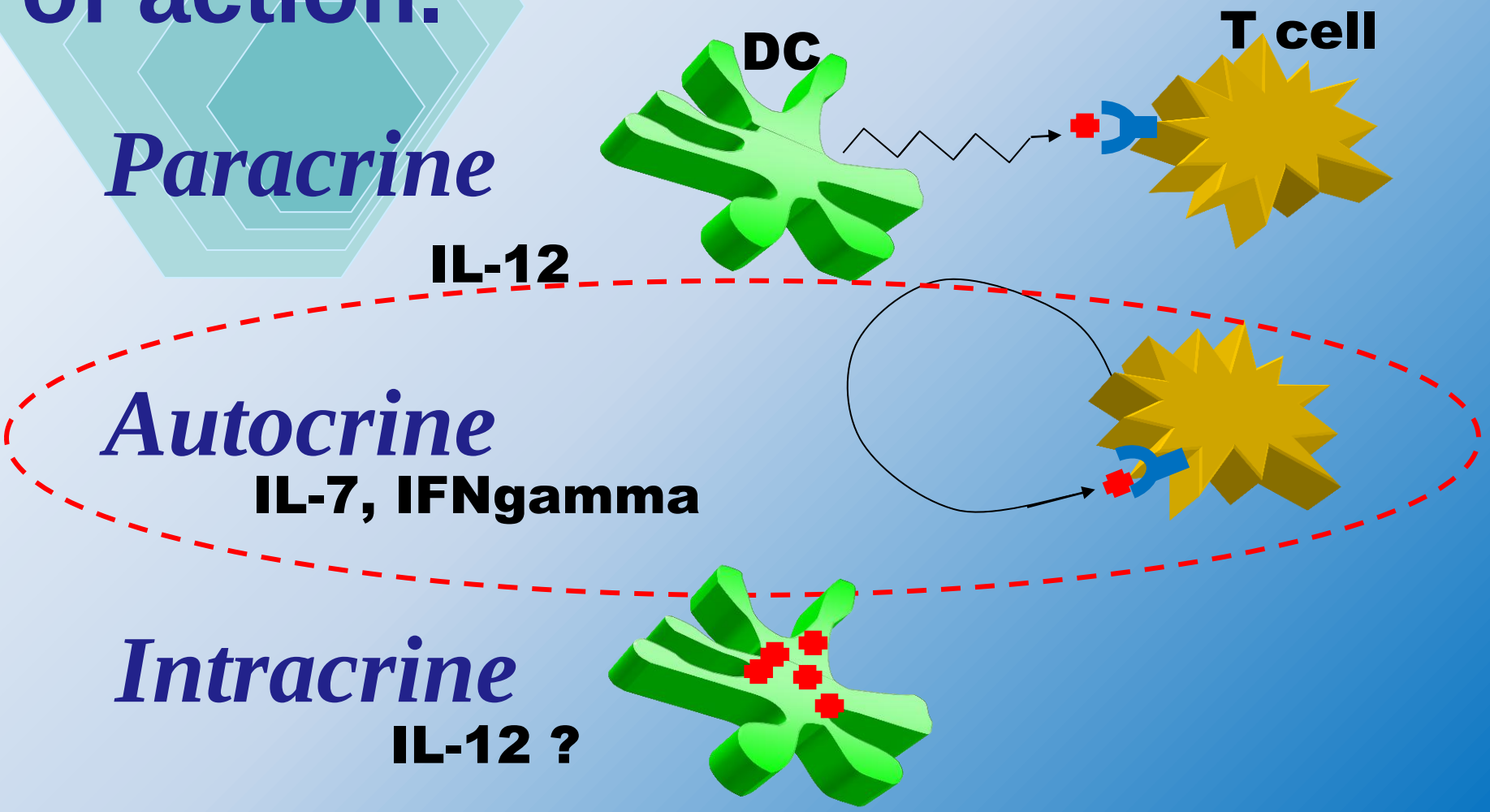
IL-12 ?



IL-7 gene delivery into T cells generates CTL populations with highest killing abilities.



Studies of three chemokines on identifying the optimal mode of action.



Conclusions

1) IL-12 gene delivery into DC generates CTL populations with highest killing abilities, more than into T cells.
This suggests that the primary mode of action of IL-12 is on DC.

2) Exogenous IL-12 protein addition did not mimic the IL-12 gene delivery in enhancing CTL killing.

3) Effect of exogenous IL-12 protein addition did not equal effect to that of AAV/IL-12 gene addition.
This is in spite of DC expressing IL-12R.
Thus, these data are most consistent with an intracellular "intracrine" activity for IL-12 which does not utilize the IL12R.

Attempts to improve antigen performance

1) Most tumor antigens contain only a few dominant MHC Class I-displayed epitopes
2) These epitopes are usually 9-11 amino acids
3) The remaining parts of the antigenic protein are "junk", of low immunologic importance.
4) Can we make a synthetic antigen gene of "concentrated" dominant epitope(s)?
5) Can such a synthetic epitope antigen gene be effective in stimulating antigen-specific CTL which effectively kill tumor cells?
6) How will such a synthetic antigen gene compare to the original, full length antigen, for CTL stimulation?

ANTIGEN PROCESSING and DOMINANT EPITOPES

Full length amino acid sequence of protein

proteinases

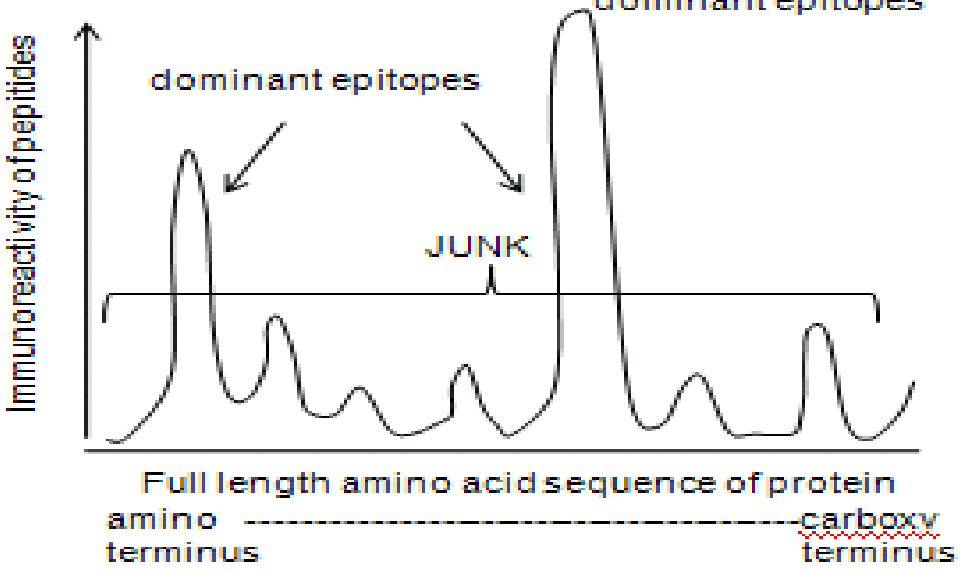
9-11 amino acids

Peptide derived from breakdown of the antigen

Display on Class I and Class II MHC molecules

Recognition by Responder T cells

CTL responders recognize, Kill, targets with dominant epitopes



HLA.A2-restricted PSA epitopes

Table 1. Binding of human prostate-specific antigen (PSA) peptides to HLA-A2 molecules

Peptide	Amino acid position in PSA	Sequence	Predicted binding to HLA-A2*	T2 binding†	
				Experiment 1	Experiment 2
PSA-1	141-150	FLTPKKLQCV	POS	516.2	688.6
PSA-2	146-154	KLQCVDLHV	POS	583.8	456.5
PSA-3	154-163	VISNDVCAQV	POS	502.8	432.7
PSA-4	29-37	VLVHPQWVL	POS	488.1	ND
PSA-5	16-25	VLVASRGRAV	POS	ND	215.2
PSA-6	42-50	CIRNKSVIL	POS	180.0	ND
PSA-7	48-56	VILLGRHSL	POS	ND	204.1
PSA-8	75-83	PLYDMSLLK	NEG	ND	188.6
PKA-1‡		FSFPDDLQCV	POS	388.3	ND
PKA-3		ILPNDECEKA	NEG	129.7	ND
HGK-1§		FLRPRSLQCV	POS	340.7	ND
HGK-3		LLSNDMCARA	NEG	127.5	ND
CAP-1‖		YLSGANLNL	POS	579.8	879.0
No peptide				195.0	209.1

*Predicted binding on the basis of reported motifs (*35*); POS = positive; NEG = negative.

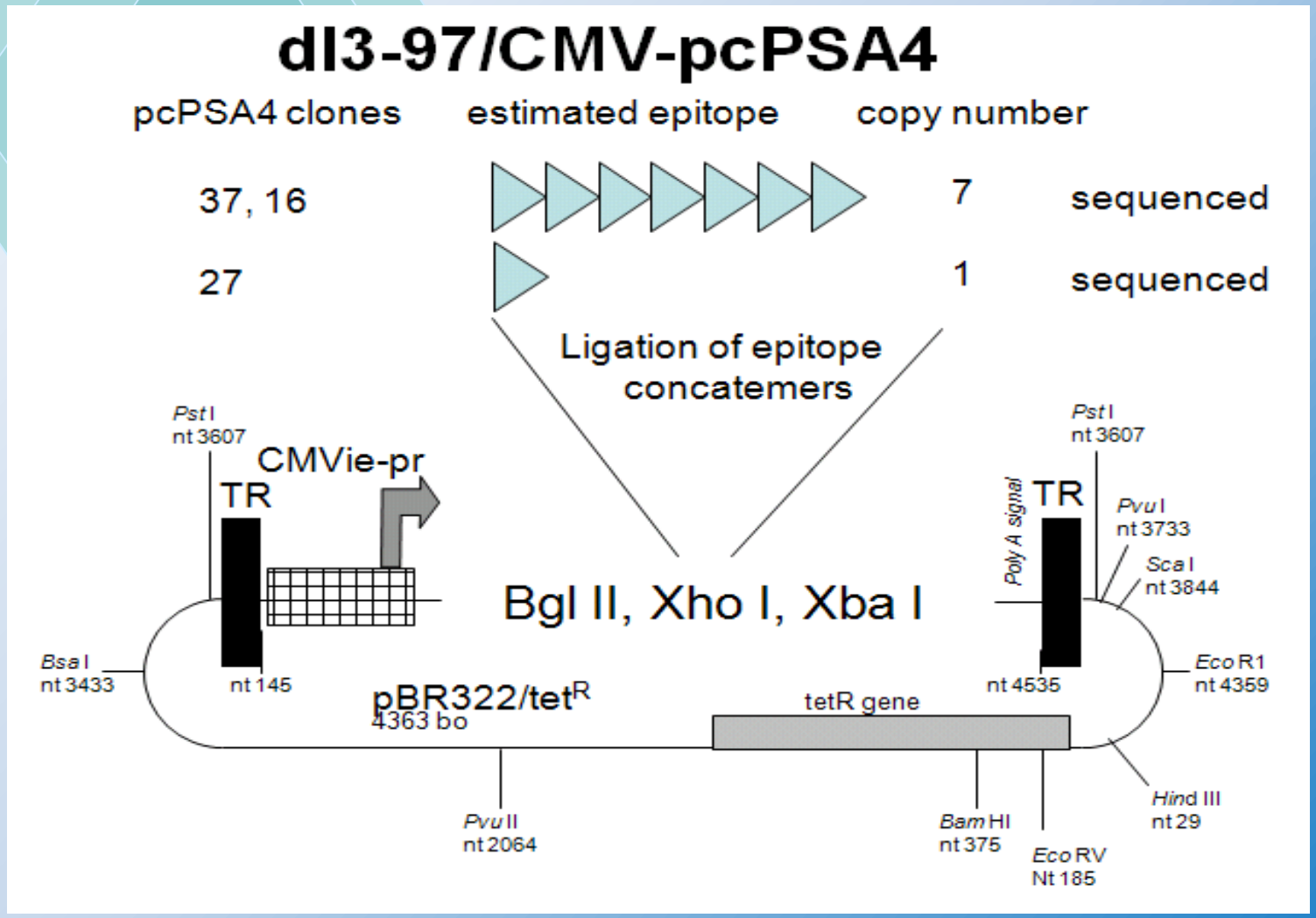
†Results are expressed in relative fluorescence values (250 was arbitrarily chosen as a cutoff value for positive). ND = not done.

‡PKA = human pancreatic kallikrein.

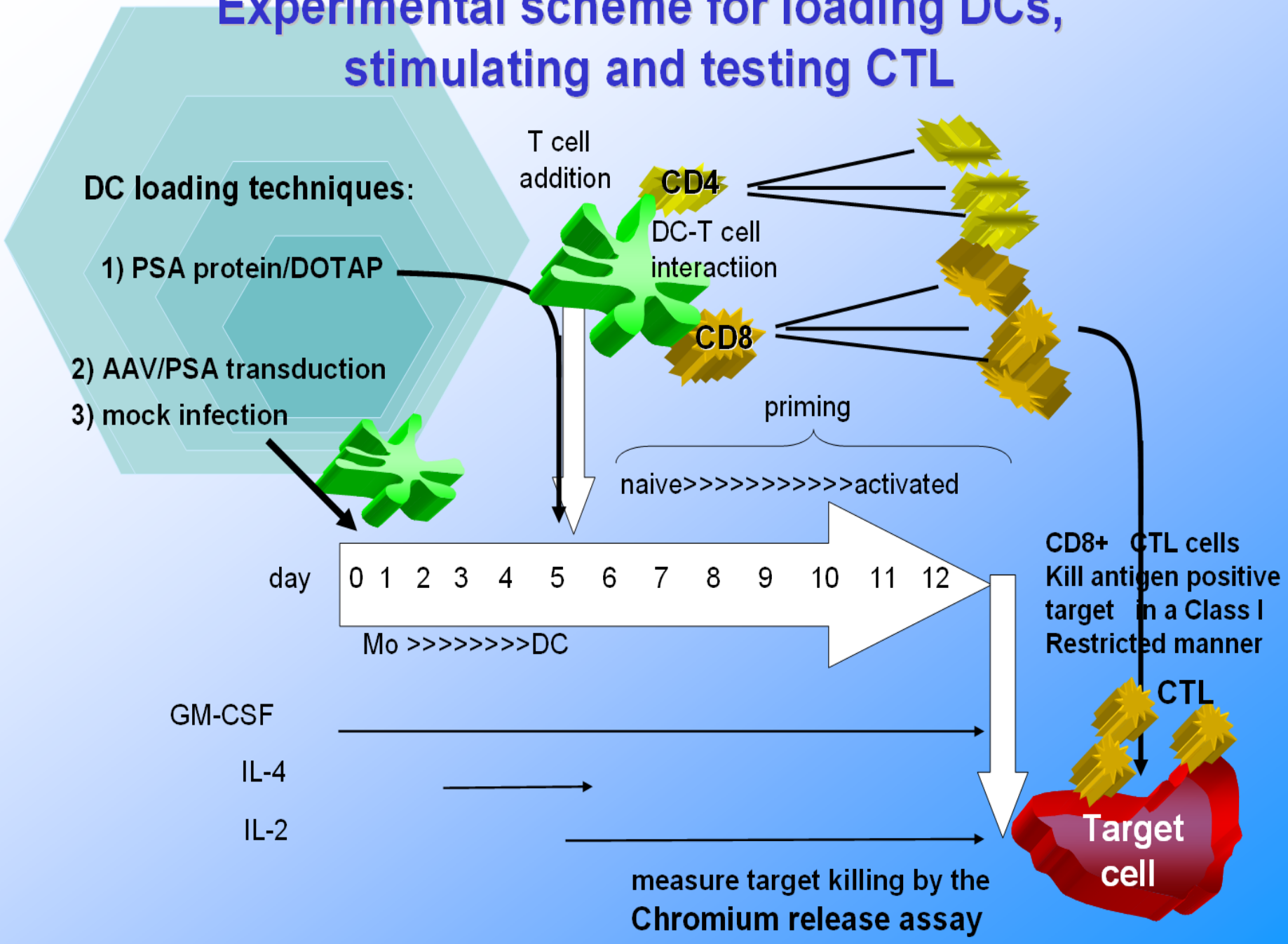
§HGK = human granulocytic kallikrein.

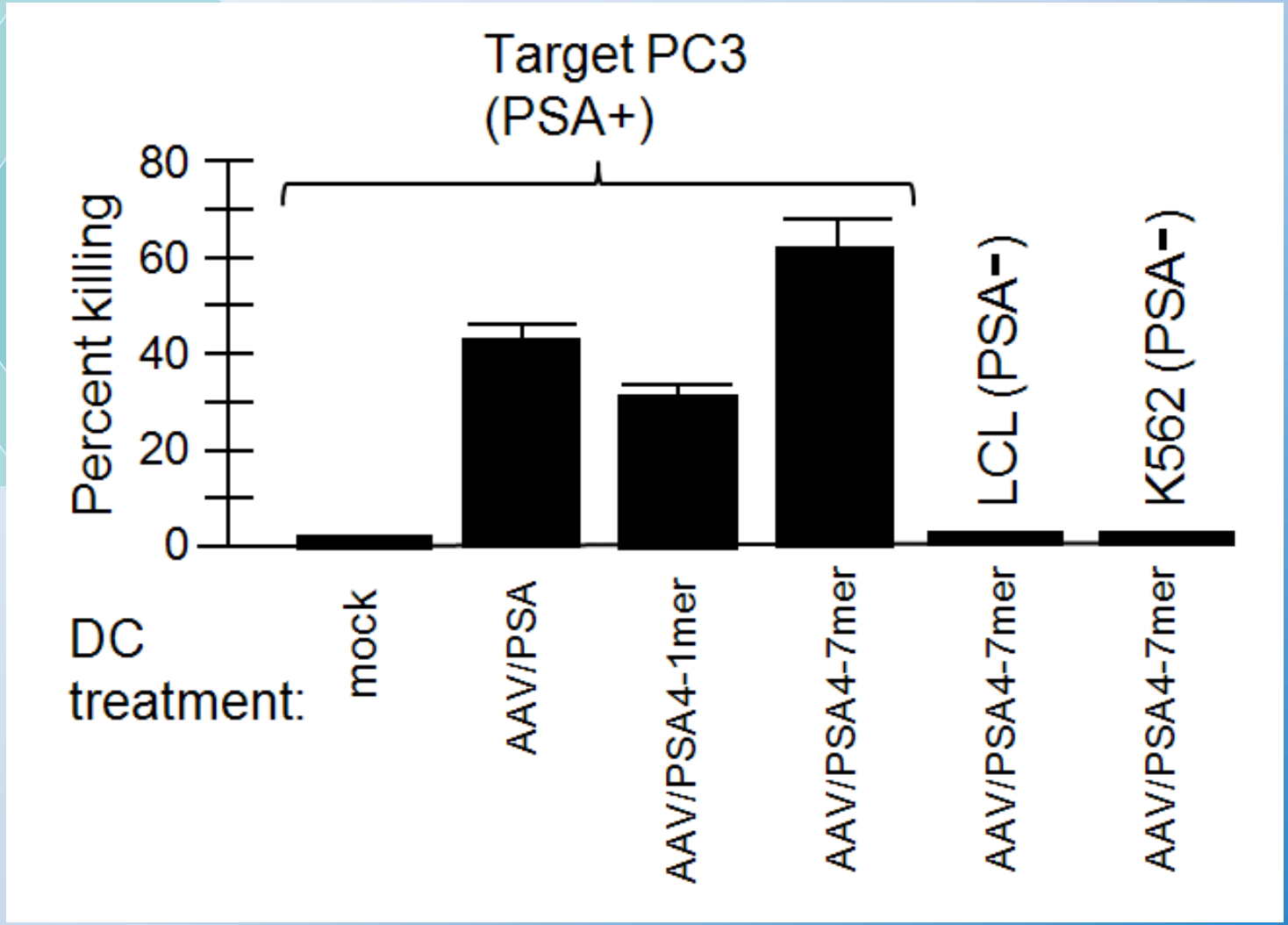
‖CAP-1 is an HLA-A2-binding carcinoembryonic antigen peptide that was used as a positive control.

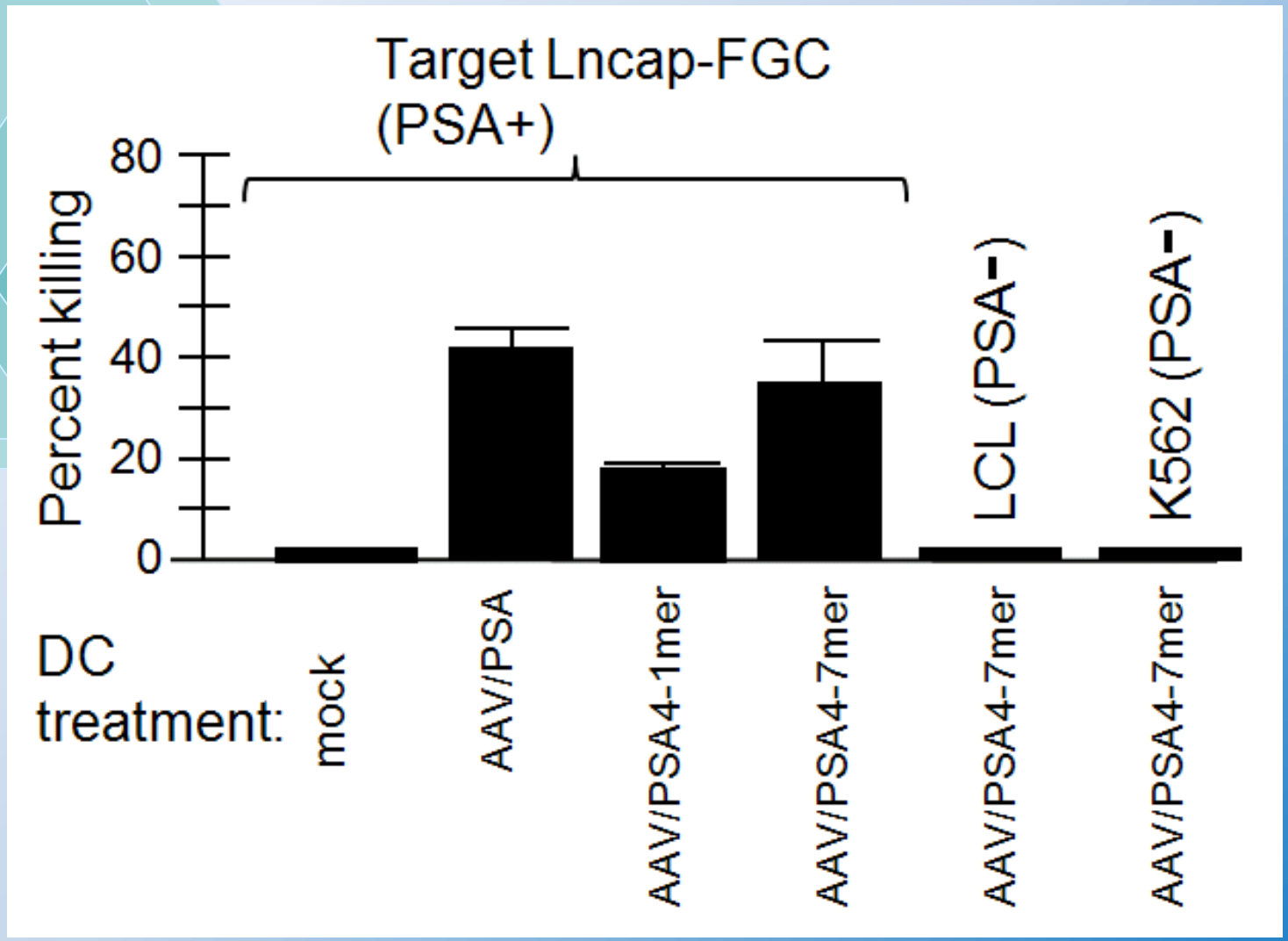
Dominant epitope PSA4 was converted from amino acid sequence into a DNA sequence, repeated multiple times and cloned, as a synthetic antigen gene, into AAV.



Experimental scheme for loading DCs, stimulating and testing CTL




Target PC3
(PSA+)
Percent killing
80
60
40
20
0
DC
treatment:
mock
AAV/PSA
AAV/PSA4-1mer
AAV/PSA4-7mer
AAV/PSA4-7mer
AAV/PSA4-7mer
LCL (PSA-)
K562 (PSA-)


Target Lncap-FGC
(PSA+)
Percent killing
80
60
40
20
0
DC
treatment:
mock
AAV/PSA
AAV/PSA4-1mer
AAV/PSA4-7mer
AAV/PSA4-7mer
LCL (PSA-)
AAV/PSA4-7mer
K562 (PSA-)

Answering the questions:

4) Can we make a synthetic antigen gene of "concentrated" dominant epitope(s)?

YES

Repeated epitope-dominant (RED) approach

5) Can such a synthetic epitope antigen gene be effective in stimulating antigen-specific CTL which effectively kill tumor cells?

YES (preliminary data)

6) How will such a synthetic antigen gene compare to the original, full length antigen, for CTL stimulation?

Preliminary data suggests that repeated epitope-dominant (RED) approach may be useful for generating enhanced synthetic antigen genes. This may lead to significantly improved immunotherapy against cancer.

Conclusions:

1) AAV-based antigen gene loaded DC stimulate Robust antigen-specific CTL which can effectively kill tumor cells.

2) AAV-based Th1 cytokine gene delivery into DC or T cells improves these antigen-specific CTL which can even more effectively kill tumor cells.

3) Other AAV types may be even better than AAV type2 In delivering genes into DC and T cells, and for DC activation.

4) Synthetic, custom, antigen gene composed of dominant epitopes is a major area of research for further improving anti-cancer CTL.

Conclusions:

1) IL-7 gene altered T cells secreted more 4X more IL-7 than equivalent modified DC.

2) IL-7 delivery and expression into T cells resulted in CTL populations with highest killing abilities. Thus, the primary mode of action of IL-7 is on the T cell.

3) In contrast IL-2 gene delivery resulted in reduced CTL killing.

4) These data are most consistent with an "autocrine" activity for IL-7 on the T cell itself.

5) It is known that the IL-7Ralpha is down-regulated after antigen stimulation. Is IL7-Ra the bottleneck?

Conclusions:

1) AAV-based immuno-gene therapy has a bright future for:

   Antigen gene delivery

   Cytokine gene delivery

   receptor gene delivery

   specialized gene delivery

2) AAV is a BSL1 agent

3) AAV has relatively low immunogenicity

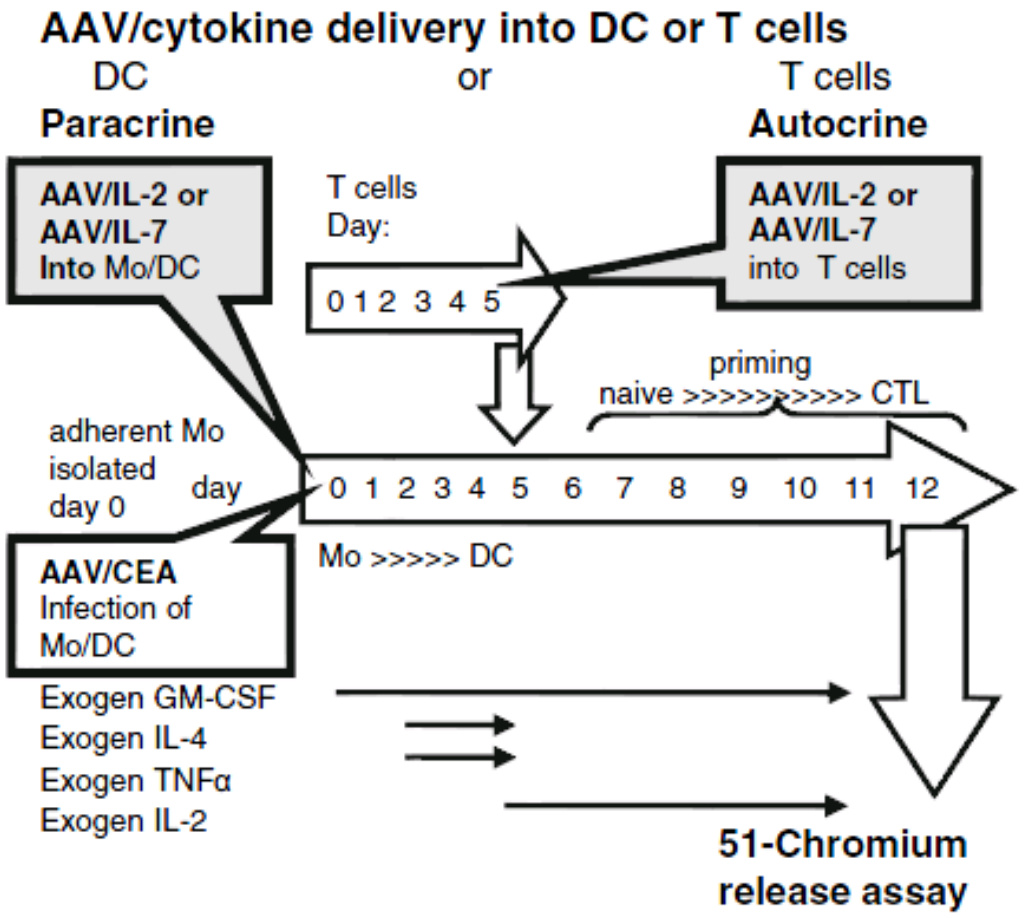


Fig. 1 Structure of the cell treatment protocol. This figure shows the temporal treatment of the Mo/DC and T cells and is self-descriptive. However, note that AAV/cytokine vectors are used to infect Mo/DC at day zero, or naive T cells just prior to co-incubation with AAV/antigen-loaded DC on day 5

Secretion of the gene delivered cytokine.

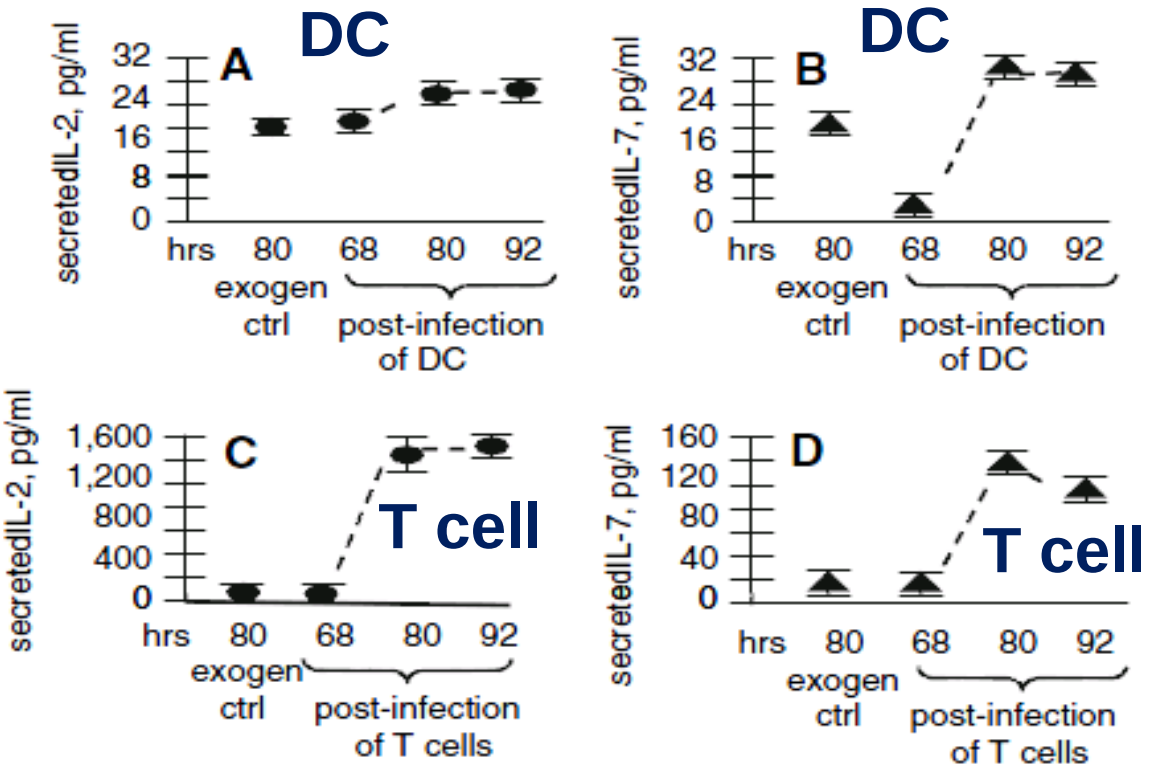


Fig. 2 Secretion of IL-2 and IL-7 in AAV-transduced cells over time. **a** Secretion of of IL-2 from transduced DC by ELISA assay. **b** Secretion of IL-7 from transduced DC by ELISA assay. **c** Secretion of of IL-2 from transduced T cells by ELISA assay. **d** Secretion of of IL-7 from transduced T cells by ELISA assay

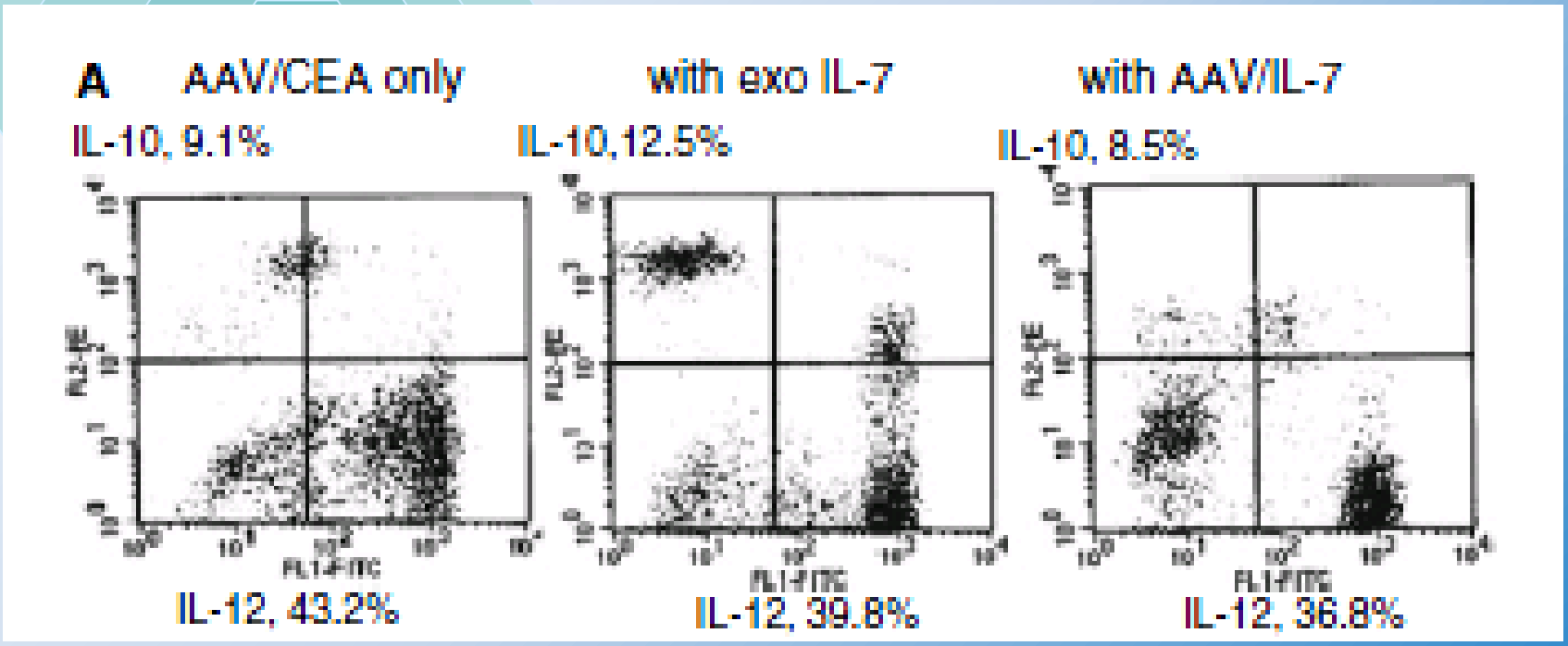
4X higher than DC

Characterization of DC.

Table 1 Surface expression of CD molecules on DC after indicated treatments

	CD14	CD40	CD80	CD83	CD86	HLA-DR
Mock	22.3	25.9	31.1	26.9	68.9	95.4
AAV/CEA	12.6	45.6	59.8	46.8	82.4	96.6
AAV/CEA + Exo-IL-2	18.1	39.2	54.3	39.7	78.1	95.9
AAV/CEA + AAV/IL-2	18.5	49.3	60.6	50.6	85.7	97.1
AAV/CEA + Exo-IL-7	17.2	38.5	57.9	48.4	89.5	91.2
AAV/CEA + AAV/IL-7	17.4	47.3	62.8	42.5	84.0	96.1

IL-12/IL-10 expression ratio in DC is roughly the same for all treatments. AAV/IL-7 treatment gave little advantage.

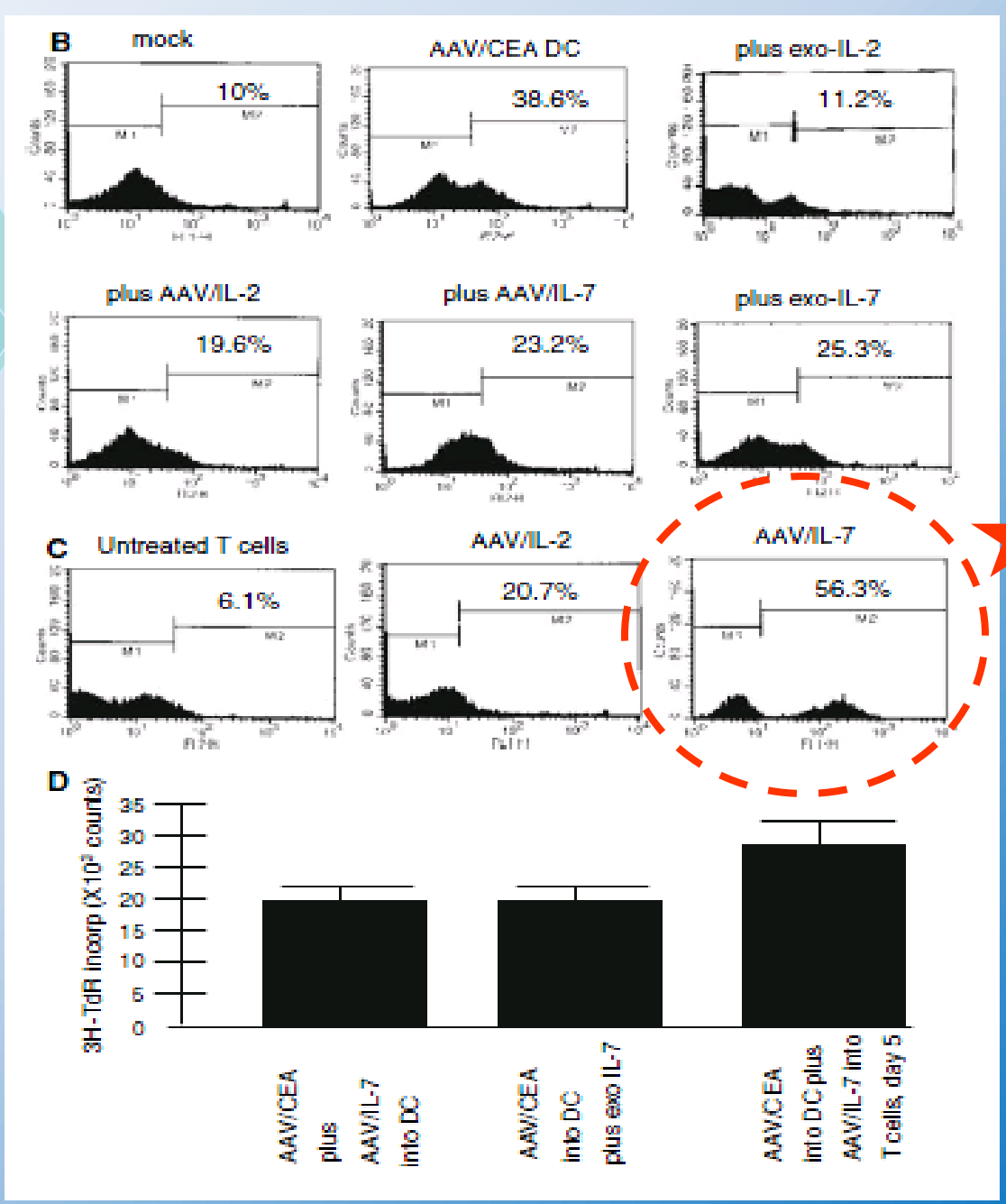


Characterization of T cells.

B: IFNgamma Expression in T cells after DC treatment.

C: IFNgamma, Expression after T cell treatment.

D: T cell proliferation after IL-7/T cell treatment.



IL-7 gene delivery into T cells generates T cell populations with best CTL characteristics.

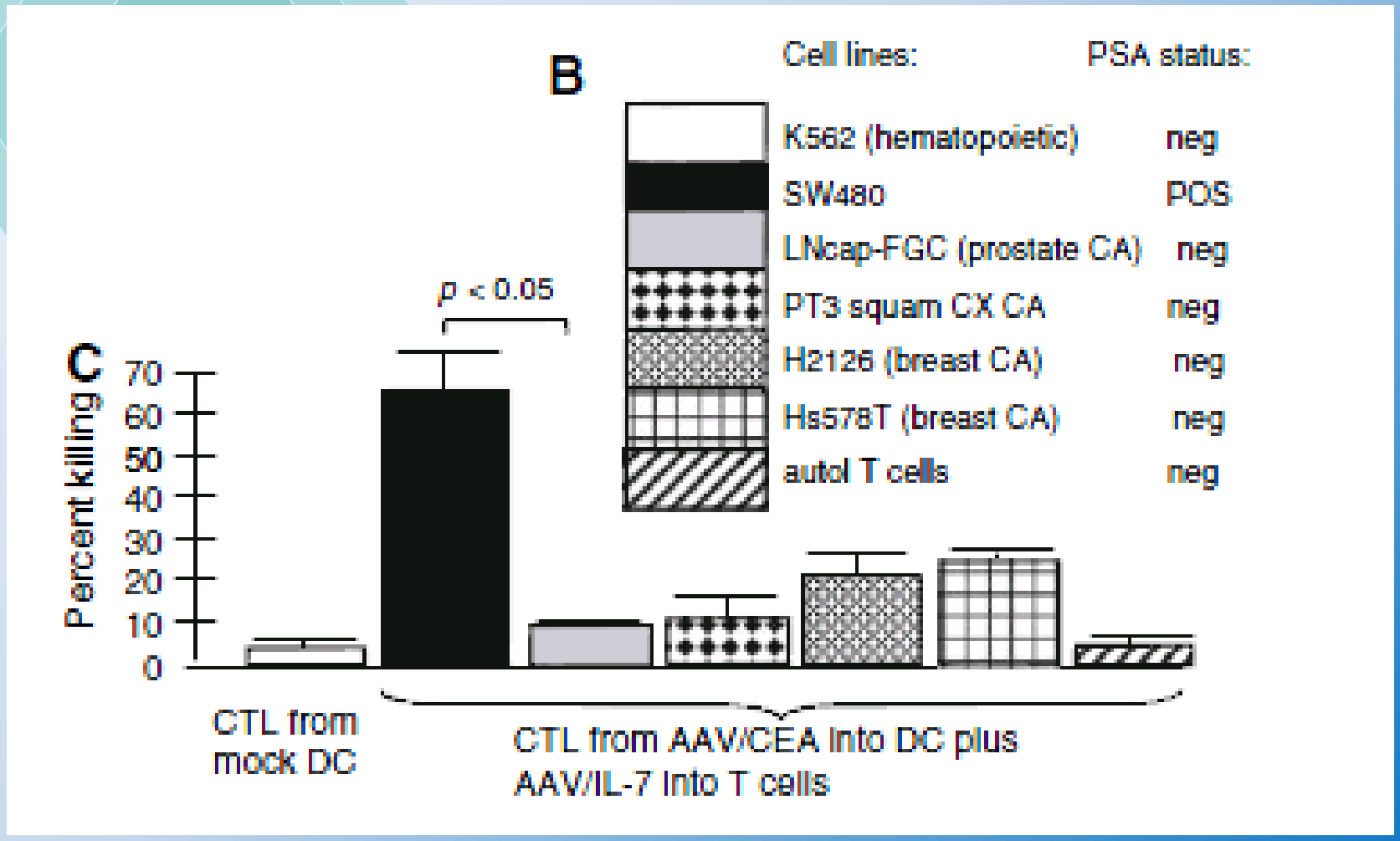
Treatment of DC:

T cells:

Table 2 (a) Immune phenotype of T cell subsets induced by rAAV-infected DC (percentage, data from FACS analysis); (b) immune phenotype of AAV/cytokine-infected T cell subsets induced by AAV/CEA-infected DC (percentage, data from FACS analysis)

	CD8/CD4	CD69, CD8	CD25, CD4
(a)			
Mock	29.4/47.9	23.8	48.2
AAV/CEA	48.6/27.9	61.4	18.4
AAV/CEA+Exo-IL-2	17.2/39.7	09.7	29.1
AAV/CEA + AAV/IL-2	20.4/42.5	11.9	33.2
AAV/CEA + Exo-IL-7	36.5/40.6	48.7	32.9
AAV/CEA + AAV/IL-7	41.3/48.1	51.6	35.8
(b)			
Uninfected	13.8/71.5	17.0	52.9
AAVIL-2	35.5/18.9	32.2	43.7
AAV/IL-7	74.9/21.4	64.5	25.1

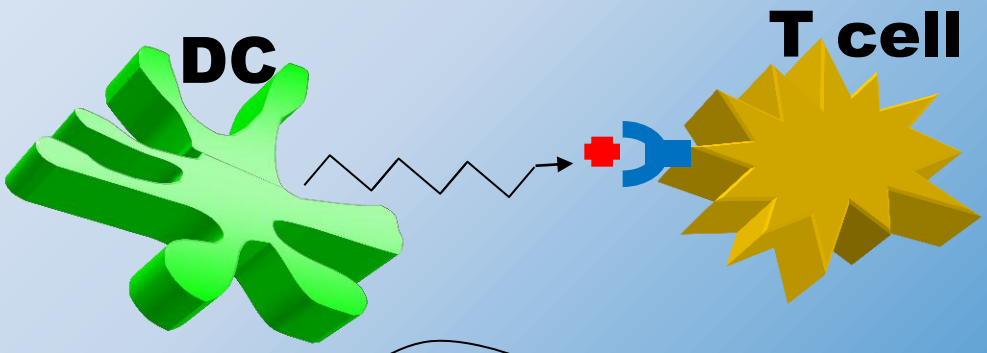
IL-7 gene enhanced CTL still kill in an antigen specific (CEA) manner.



Studies of three chemokines on identifying the optimal mode of action.

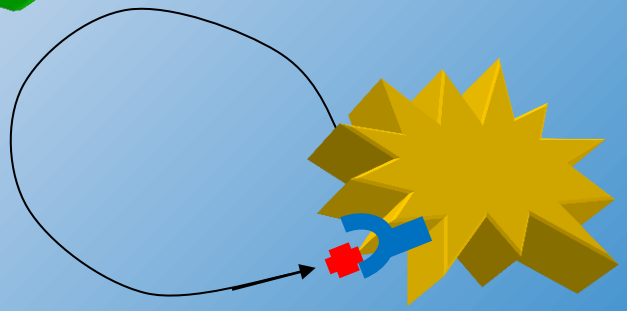
Paracrine

IL-12



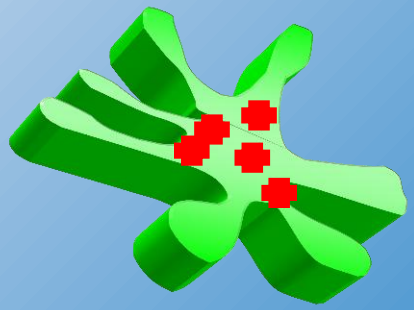
Autocrine

IL-7, IFNgamma



Intracrine

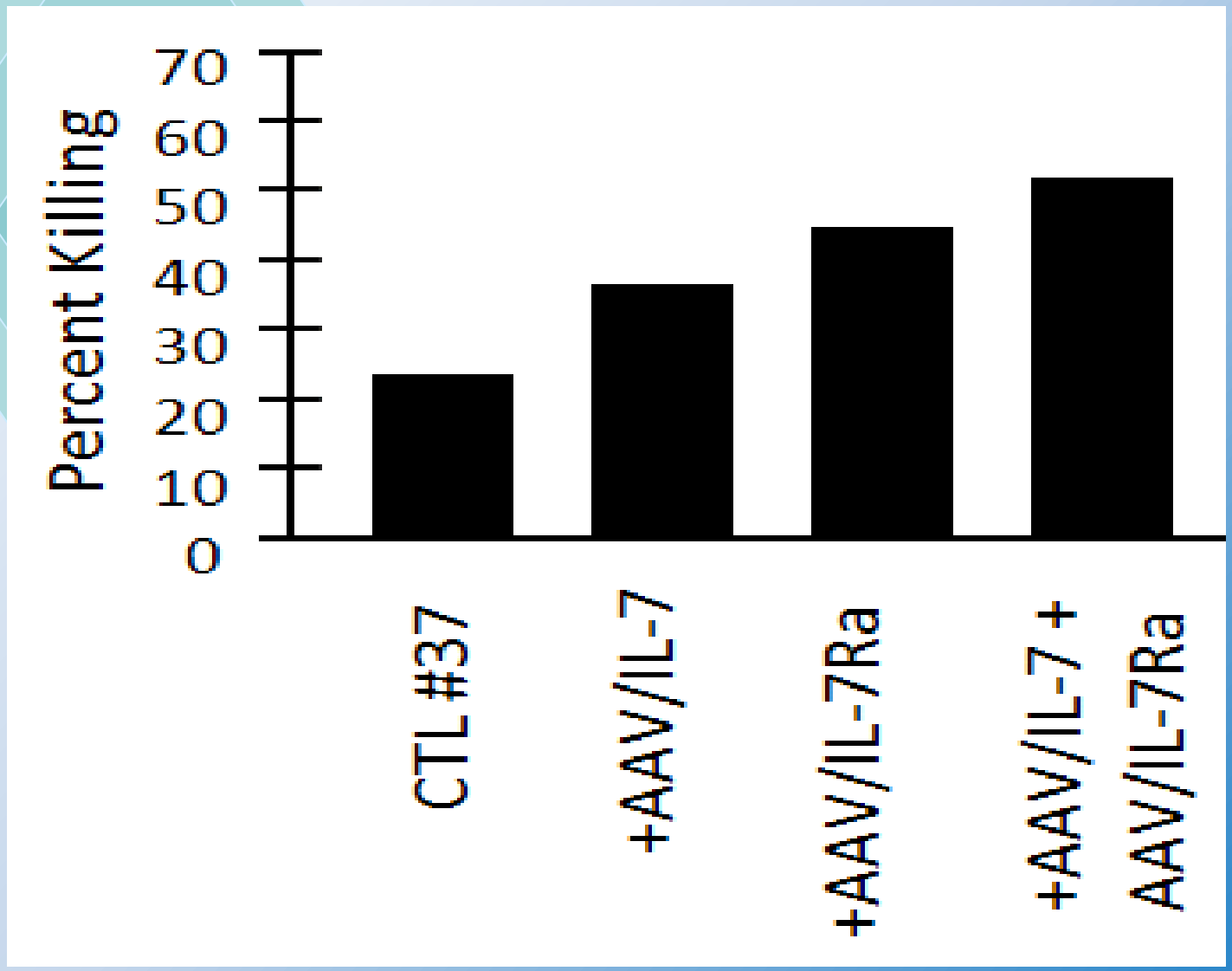
IL-12 ?



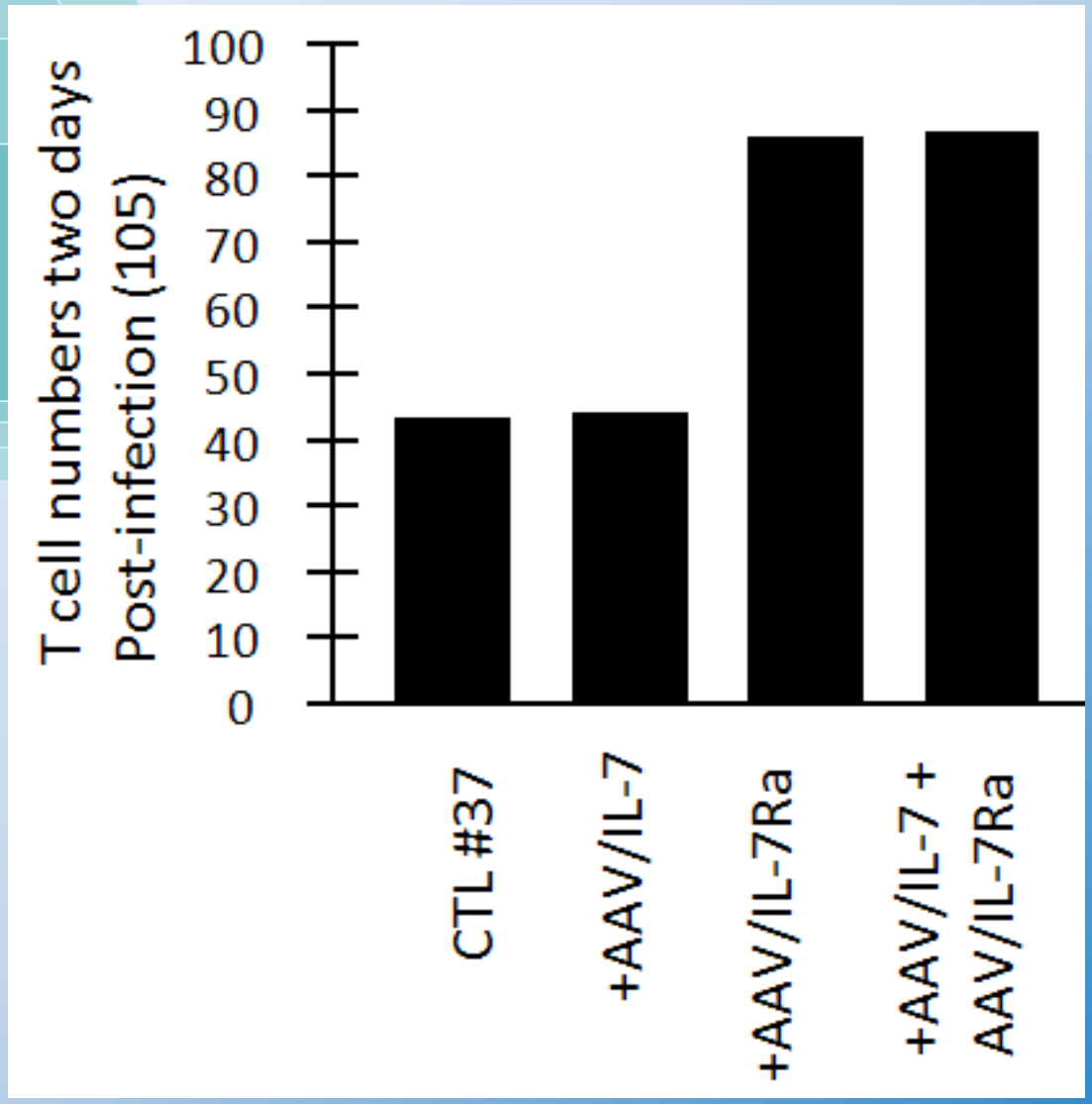
Dual cytokine plus cytokine receptor gene delivery.

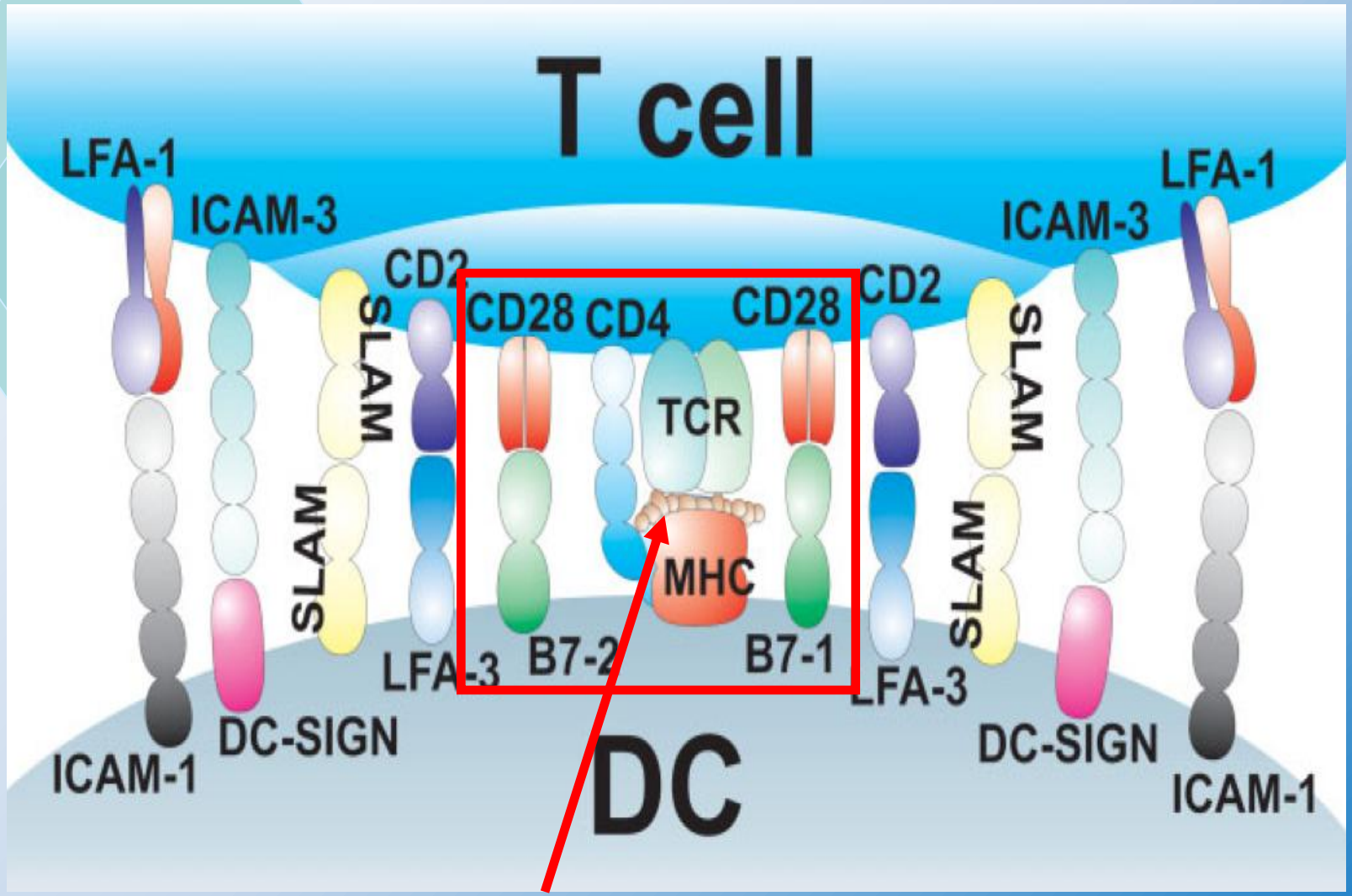
If IL-7 gene delivery into T cells generates stronger CTL killer populations, what about gene delivery of IL-7 plus its receptor IL-7Ralpha?

IL-7 gene delivery into T cells generates stronger CTL killer populations, however in this preliminary experiment IL-7Ralpha had a larger effect.

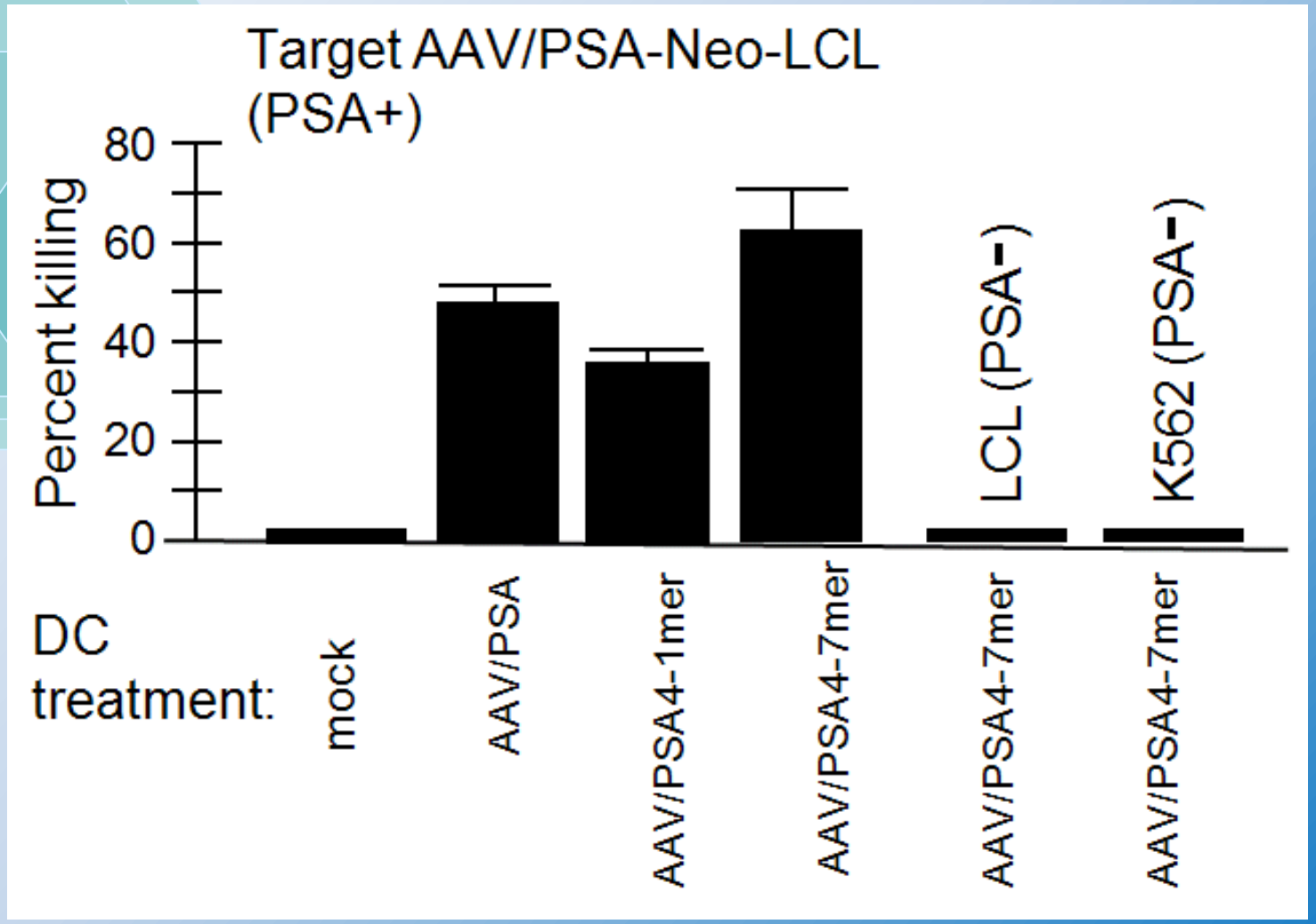


IL-7Ralpha may also stimulate higher T cell proliferation as well.

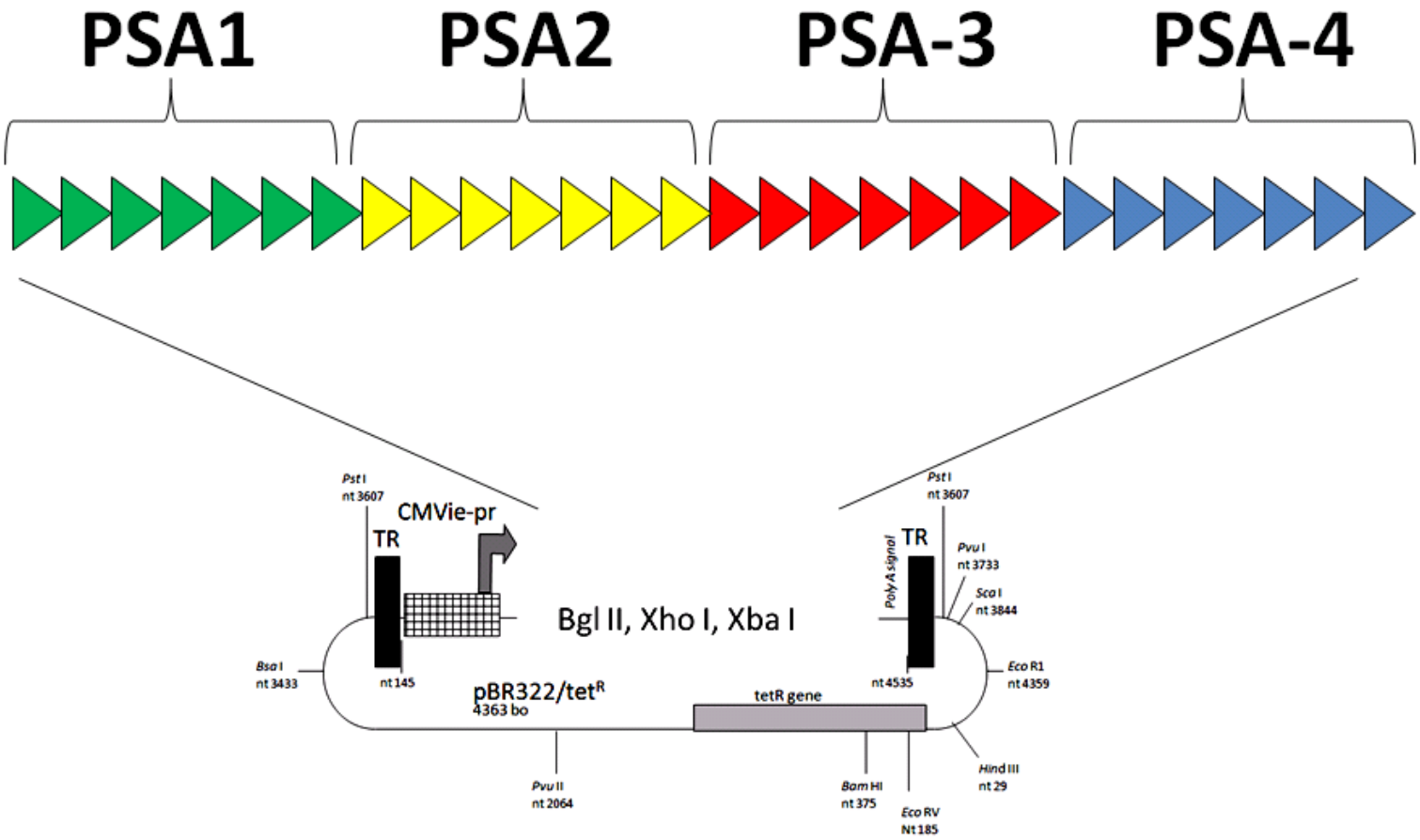




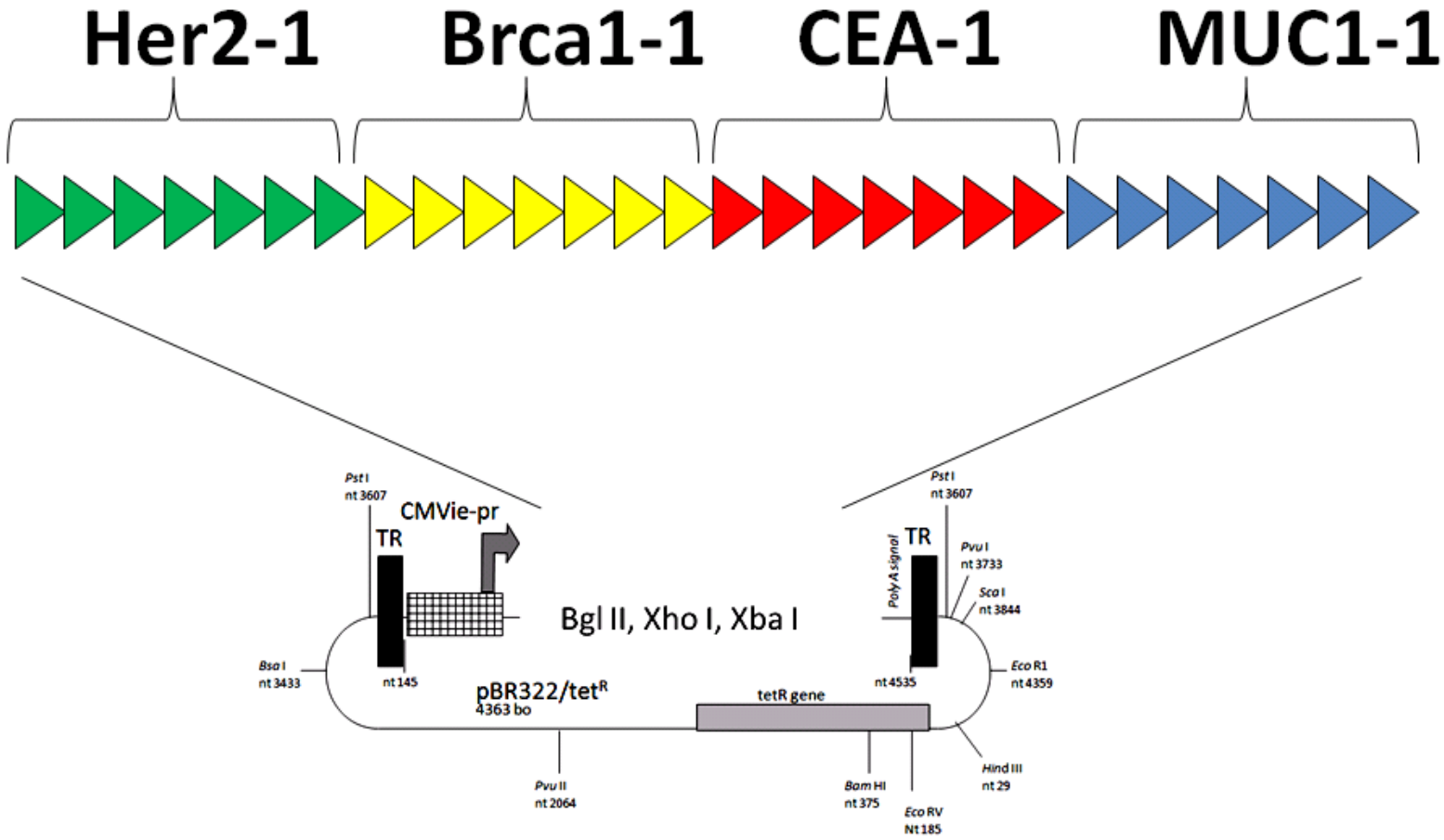
Antigenic epitope
9-11 amino acids


Target AAV/PSA-Neo-LCL
(PSA+)
Percent killing
80
60
40
20
0
DC
treatment:
mock
AAV/PSA
AAV/PSA4-1mer
AAV/PSA4-7mer
AAV/PSA4-7mer
AAV/PSA4-7mer
LCL (PSA-)
K562 (PSA-)

Super-antigen generated from multiple dominant epitopes from one antigen protein, eg. PSA. Strongly targets *one* antigen



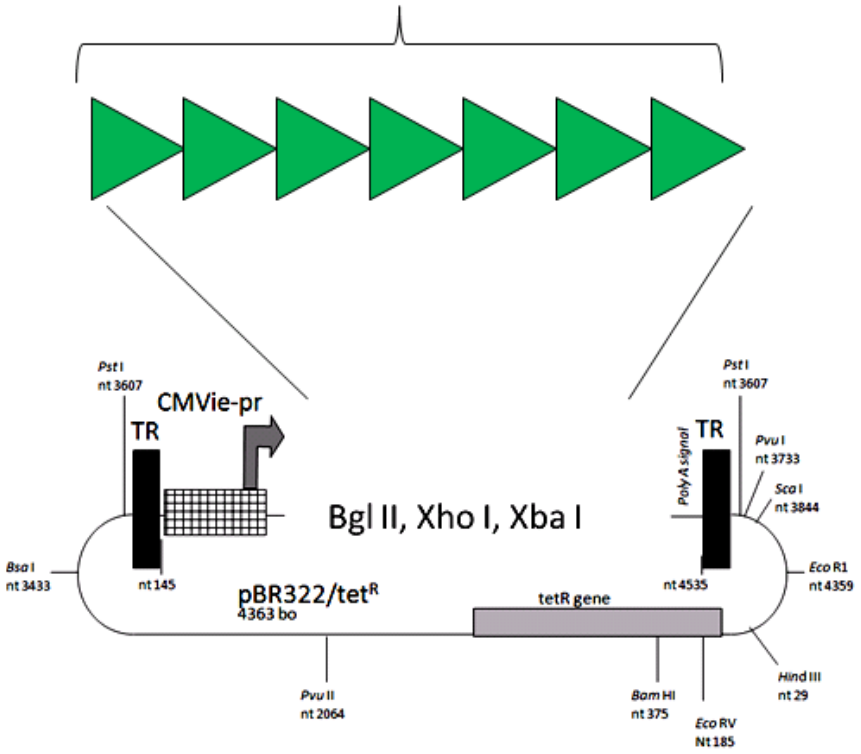
Super-antigen generated from dominant epitopes from multiple antigen proteins, eg. PSA. Targets *multiple* antigens, prevents "escape" mutants from growing



Specialty antigen #1:

Enhanced antigen generated from mutant oncoprotein epitopes often present in cancer. eg. K-ras codon 12, 13 or 61.

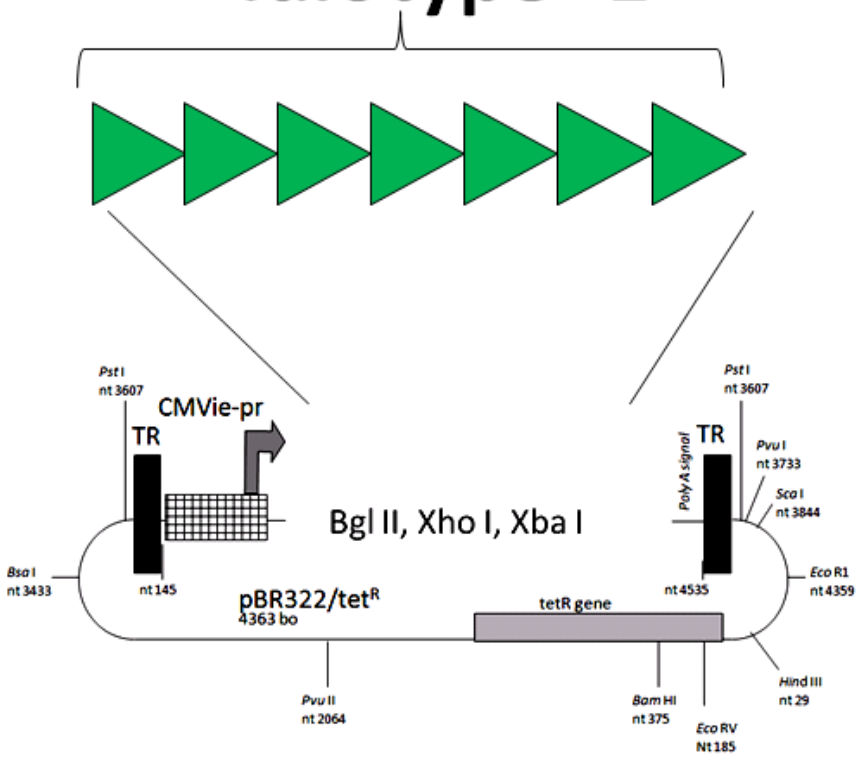
K-ras codon 12

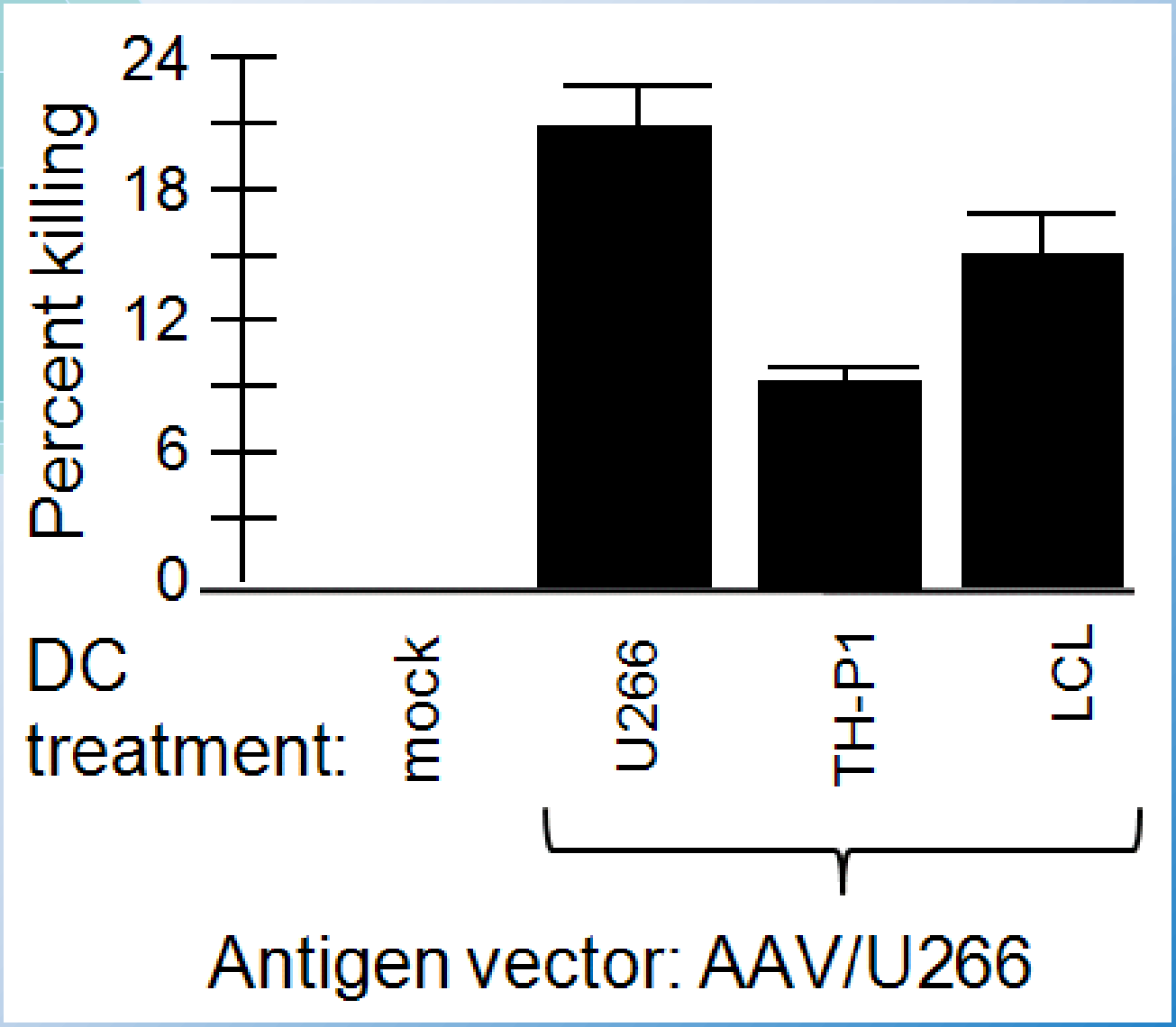


Specialty antigen #2:

Enhanced antigen generated from an important but weak antigen.

Eg. myeloma idiotype antibody hypervariable region



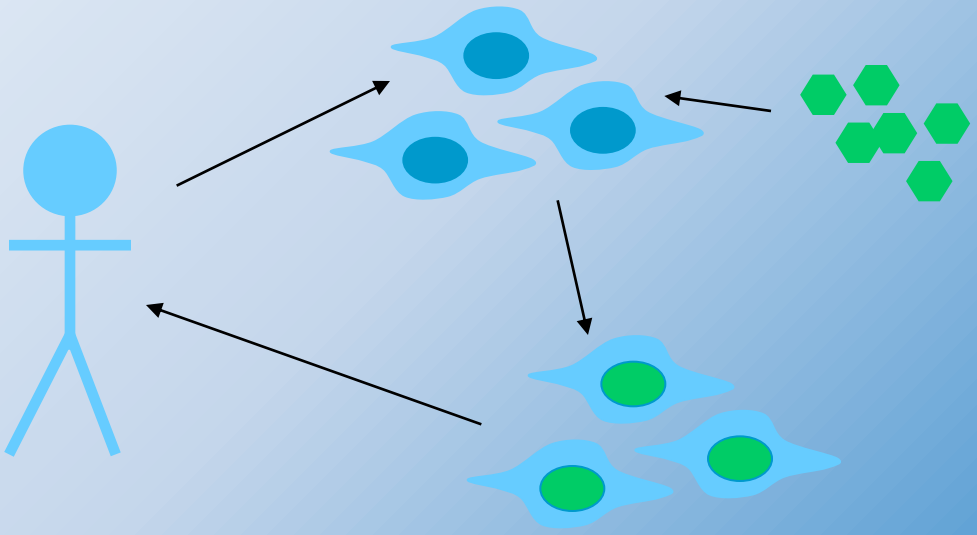

Percent killing
24
18
12
6
0
DC treatment:
mock
U266
TH-P1
LCL
Antigen vector: AAV/U266

The People

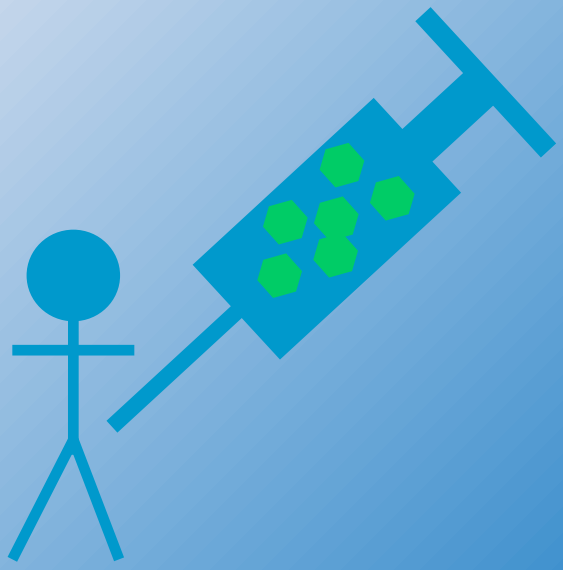

Dr. Raghu Muppidi
Dr. Maha Mahadevan
Dr. Yong Liu
Dr. Min Shi
Dr. Sarmistha Bhattacharya
Dr. Maohua Cao

General approaches of delivery

EX VIVO:

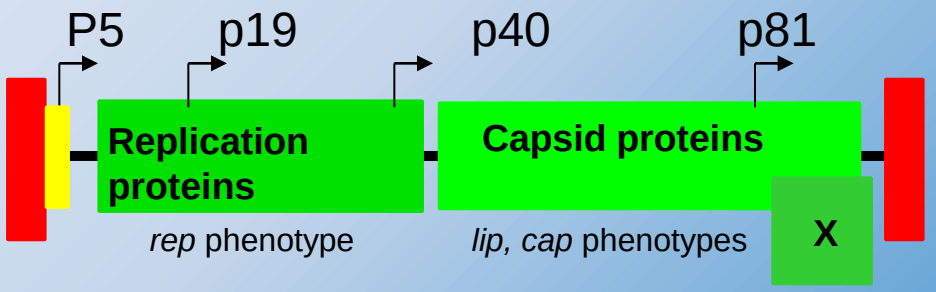


IN VIVO:



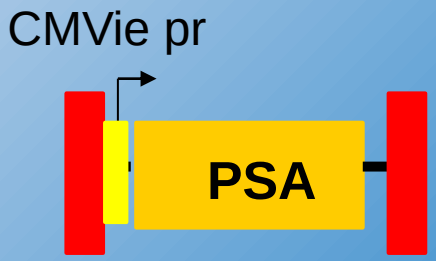
AAV/PSA vector for treating prostate cancer

wt AAV-2



AAV-2/PSA

(prostate specific antigen
Is expressed only on prostate
Cells.



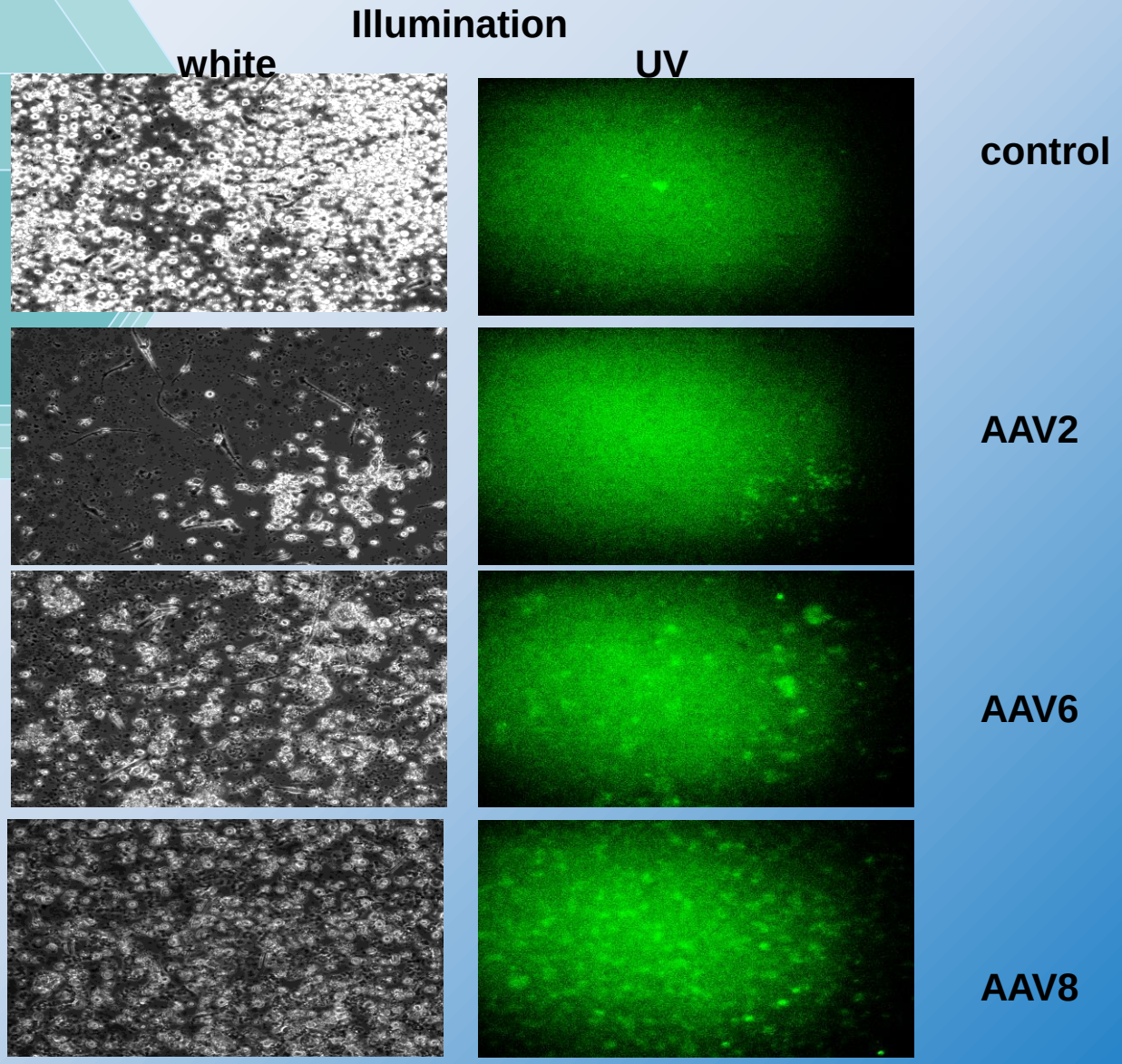
Attempts to improve antigen and cytokine gene delivery

1) We use AAV type 2, but now there are over 100 AAV types isolated.
2) Maybe other types may deliver genes better into DC and T cells.
3) Here we compare AAV types 2, 730, 6 and 8.
4) In addition to gene delivery, which AAV type actives DC best, up-regulating B71 (CD80) and B72 (CD86)?
5) What about the new AAV tyrosine mutant types by Srivastava.

Attempts to improve strength of CTL by Th1 cytokine gene delivery

1) Th1 cytokines are critical for DC antigen presentation to naïve responder T cells to generate antigen-specific CTL killers.
2) Examples of Th1 cytokines IL-7, IL-12, IFN gamma, IL-15, IL-18, IL-21, etc.
3) But which immune cell type should secrete the cytokine? DC (paracrine) or T cell (autocrine). This is an issue in immunology rarely addressed.
4) Using AAV gene delivery we can force the expression of the cytokine into whichever cell type we want.
5) This is an important issue for immuno-gene therapy.

Delivery of eGFP by various AAV types Into DC



Cancer Immunol Immunother
DOI 10.1007/s00262-009-0798-0

ORIGINAL ARTICLE

Comparison of AAV/IL-7 autocrine (T cell) versus paracrine (DC) gene delivery for enhancing CTL stimulation and function

Chang-Xuan You · Yong Liu · Min Shi · Maohua Cao · Rong-Cheng Luo · Paul L. Hermonat

Received: 17 September 2009 / Accepted: 10 November 2009

Abstract Adoptive transfer of antigen-specific cytotoxic T lymphocyte (CTL) into patients holds promise in treating cancer. Such anti-cancer CTL are stimulated by professional antigen-presenting dendritic cells (DC). We hypothesize the gene delivery of various Th1-response cytokines, highest interferon γ expression, highest CD8(+):CD4(+) ratio, highest CD8(+), CD69(+) levels, and lowest CD4(+), CD25(+) (Treg) levels. These data are consistent with higher killing by the AAV/IL-7-altered CTL. These data strongly suggest that IL-7 autocrine gene delivery is