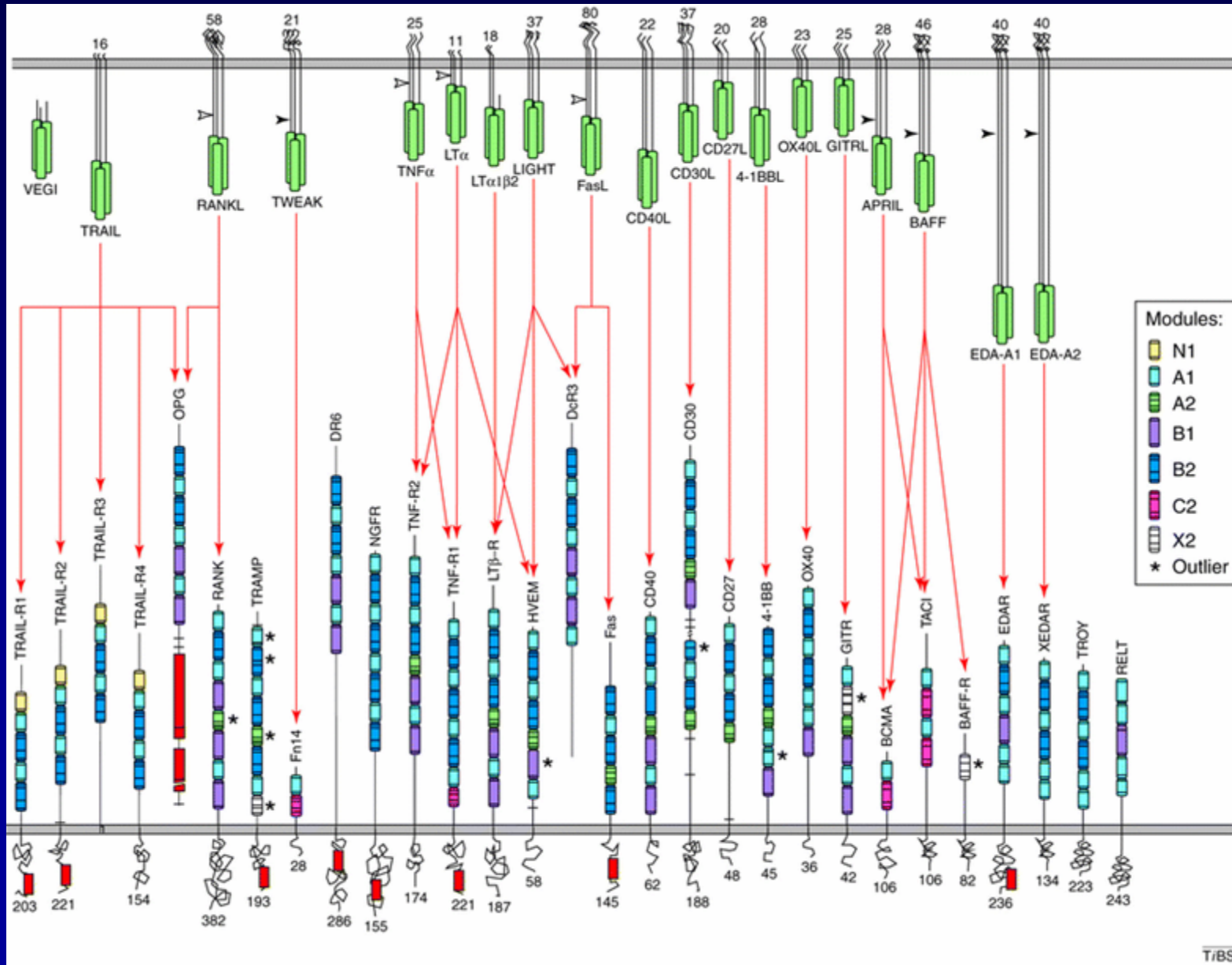


**Treatment of established tumors with
peritumoral injections of
CD40 ligand (CD40L), CpG, poly(I:C), and
extracellular ATP in murine models**

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Disclosure: Richard Kornbluth is the inventor on patents for multimeric TNFSF ligands and immunostimulatory combinations that have been assigned to the University of California San Diego

The TNF Superfamily of Ligands



Multimeric soluble TNFSF ligands

Rationale: Single trimer forms of most TNFSF ligands are not strongly stimulatory

Solution: Produce multimeric (=many trimer forms) of TNFSF ligands

Method: Fuse the extracellular domain (ECD) of TNFSFs with a multimerization scaffold from a collectin or C1q superfamily protein

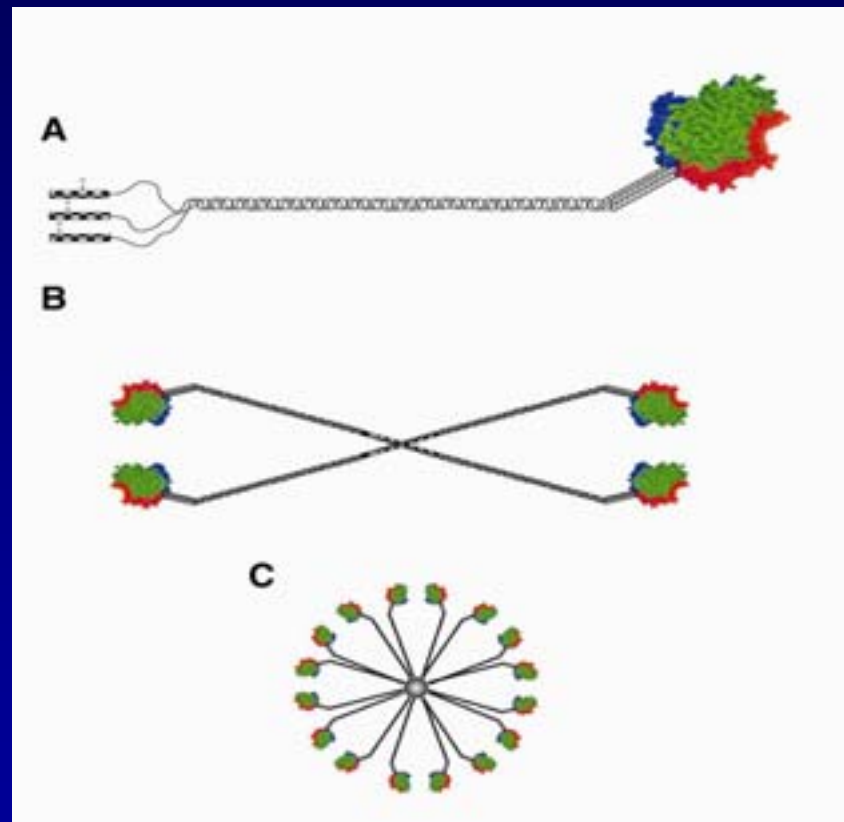
Haswell et al (Al-Shamkhani and Glennie) *Eur J Immunol* 31:3094, 2001.

Holler et al (Tschopp and Schneider) *Mol Cell Biol* 23:1428, 2003.

Kee et al (Kornbluth) in preparation

Example: SP-D-CD40L – body of pulmonary surfactant protein D with the ECD of CD40L

CD40L-SPD, a fusion protein of the extracellular domain of CD40L and the body of surfactant protein D



K. Kee and R.S. Kornbluth, in preparation

Immunologically relevant TNFSF Ligands

- ► CD40 Ligand (CD154)
- ► GITR Ligand
- LIGHT
- TRAIL
- RANK Ligand
- 4-1BB Ligand
- CD27 Ligand (CD70)
- OX40 Ligand
- BAFF
 - included in this presentation

CD40 Ligand

- Produced by activated CD4+ T cells
- Activates dendritic cells and other APCs
- Required to establish memory CD8+ cells
- Agonistic anti-CD40 antibody can cause tumor regression
- Concerns with agonistic anti-CD40
 - Toxicity
 - Immunogenicity

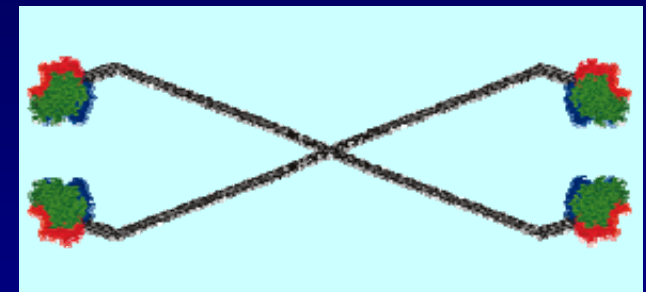
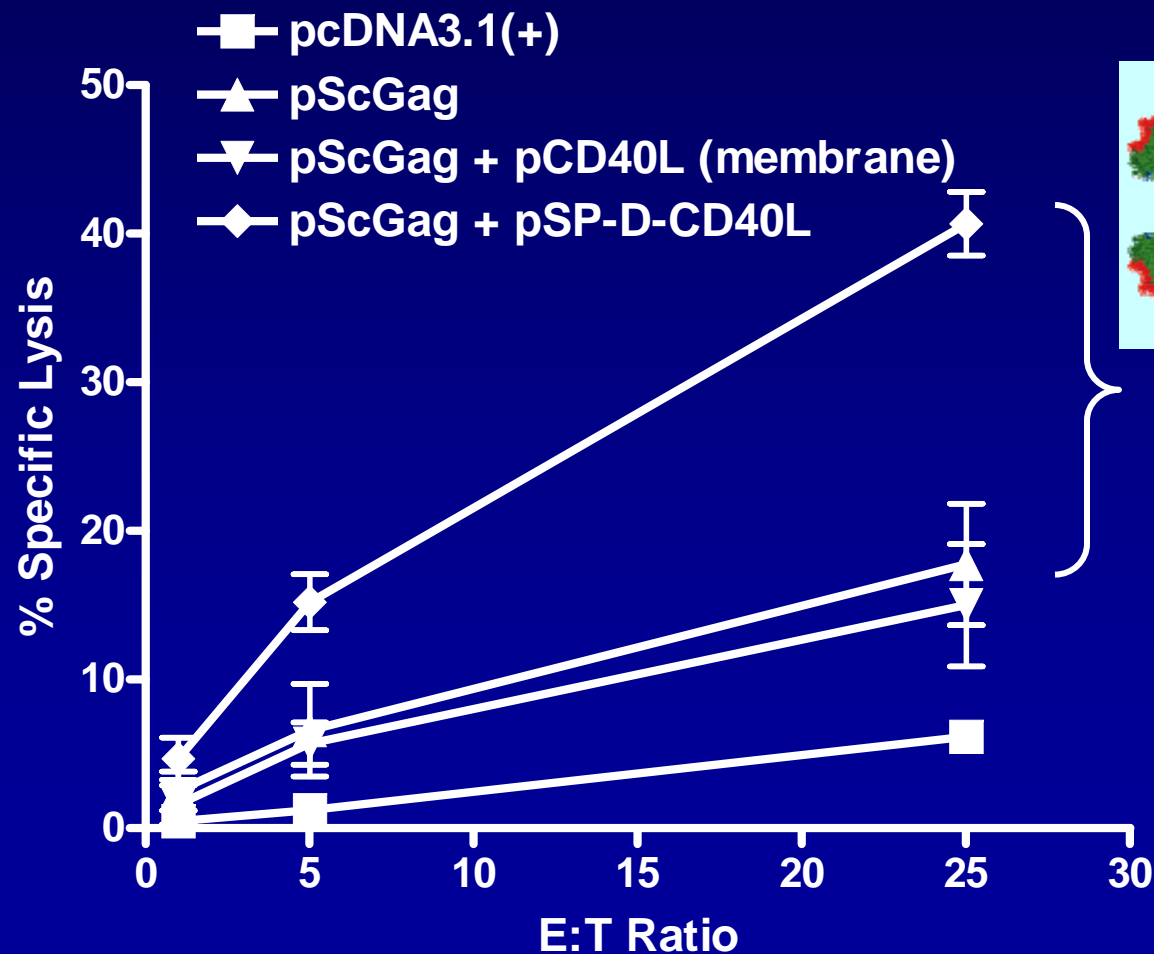
CD40 stimulation of Dendritic Cells

- Activates DCs to express CD80 and CD86 for Signal 2
- Activates DCs synergizes with TLR stimulation to produce IL-12 p70 for Signal 3
- Activates DCs to express GITRL which acts on GITR to shut off CD4+CD25+ regulatory T cells (“Tregs”)
- Requires clustering of CD40

HIV DNA Vaccine Methods

- BALB/c mice (4 or 5 mice per group)
- Inject 50 μ l i.m. in each quadriceps muscle
- 80 μ g antigen plasmid, pScGag (secreted codon-optimized Gag plasmid)
- 20 μ g adjuvant plasmid
- Single immunodominant peptide
AMQMLKETI used for pScGag CTL and
IFN- γ ELISPOT assay

pSP-D-CD40L but not membrane CD40L enhances Gag CTL activity



$p < 0.01$

CD8+ T cell IFN- γ ELISPOT responses

pcDNA3.1



pScGag



pScGag
+
pMemCD40L



pScGag
+
pSP-D-CD40L



Fresh splenocytes were plated directly into ELISPOT wells (10^6 cells/well) and stimulated with P815 cells pulsed with a single peptide epitope.

pSP-D-CD40L does not act systemically

- Mice appear healthy and have normal weight
- No evidence of splenomegaly or lymphadenopathy
- Muscle and lung histology normal
- No antibody to SP-D-CD40L detectable
- pSP-D-CD40L must be mixed with the antigen plasmid to be active (contralateral injections of pSP-D-CD40L and antigen don't vaccinate)
- Repeat vaccinations with pSP-D-CD40L and a different antigen plasmid are effective

Immunostimulatory Combinations for Tumor Immunotherapy

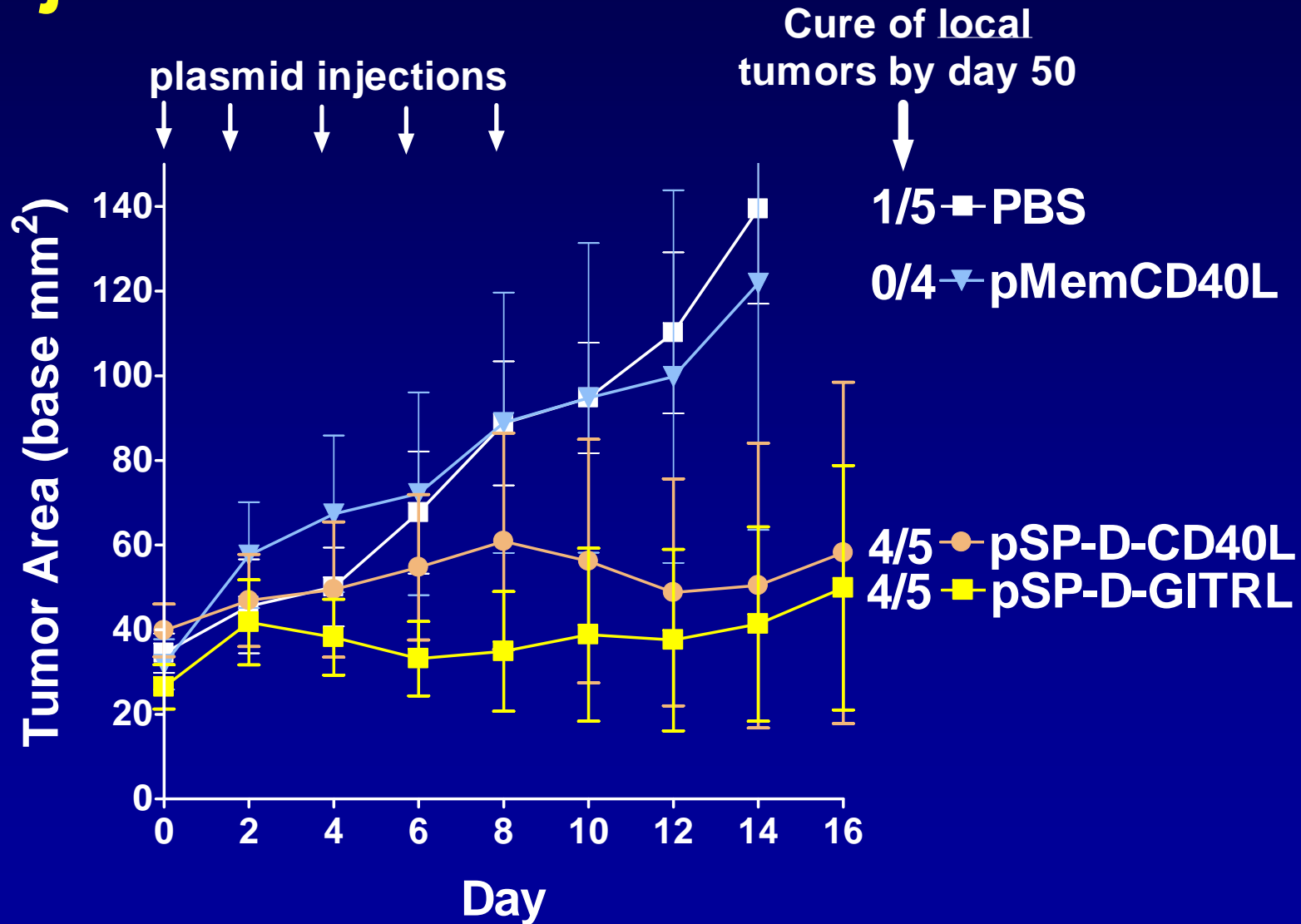
Three Routes to DC Activation

- CD40 stimulation: needed for generating long-term memory CD8+ T cells
- TLR pathways: MyD88 and TRIF pathways synergize with CD40 to stimulate DCs to produce massive amounts of IL-12 p70 (300 ng/ml)
- Inflammasome pathways: Contain pyrin, NACHT, or LRR domains. Activated by microbial products to stimulate IL-1 β release

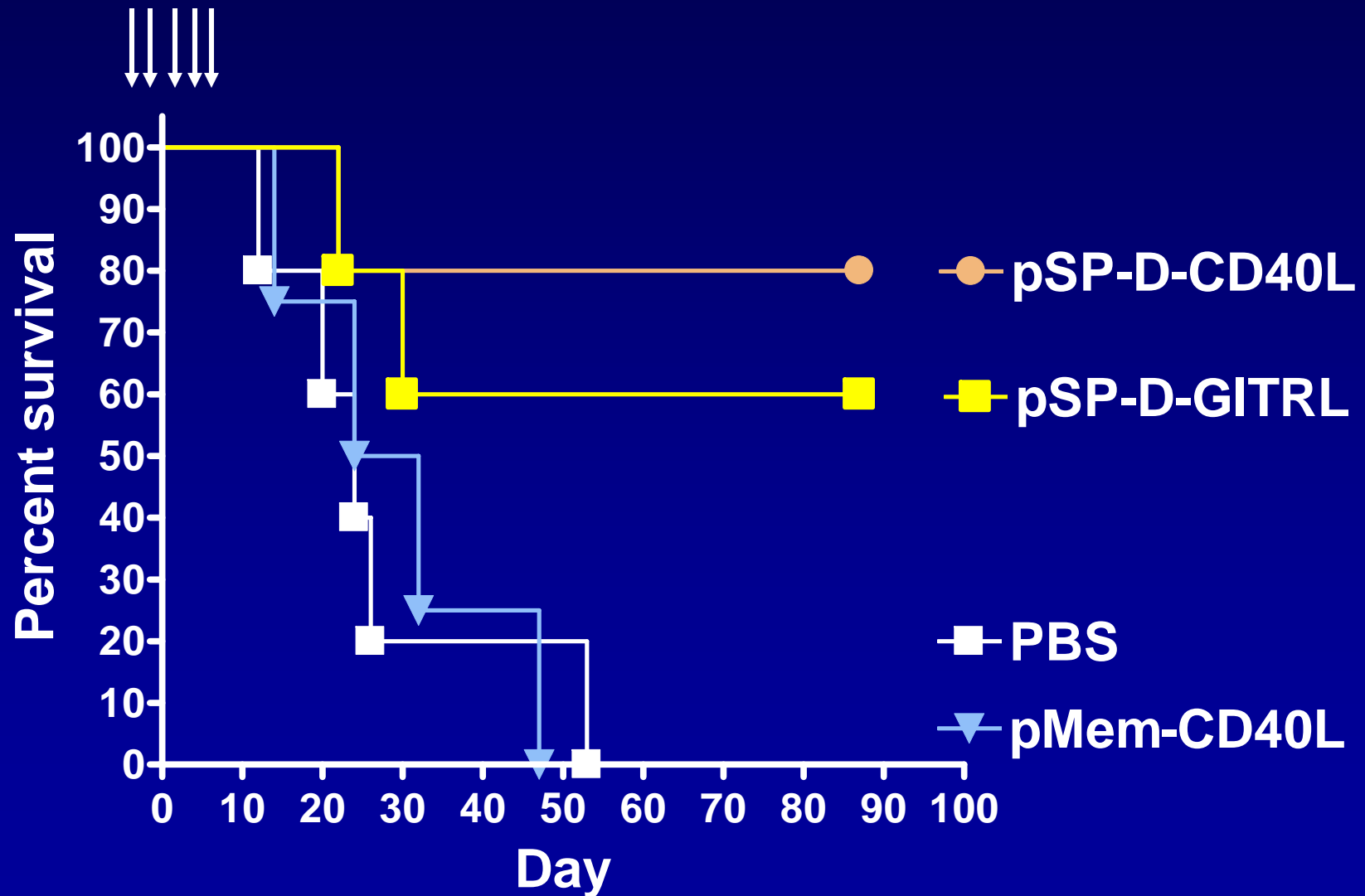
Models of Established Tumors

- A20 lymphoma is CD40^{high} and Treg-rich
- B16F10 melanoma is CD40^{lo} and not strongly affected by Treg depletion
- 5 mice per group
- 1×10^5 tumor cells injected s.c.
- When tumor >4mm (4-6 days later) begin injections
- 50 μ g plasmid DNA injected peritumorally every other day x 5

Cure of A20 lymphoma by peritumoral injection of either CD40L or GITRL



Survival benefits of CD40L or GITRL peritumoral injections



Treatment of B16-F10 melanoma requires combinations of CD40L + TLR agonists CpG and Poly(I:C) + inflammasome agonist ATPe

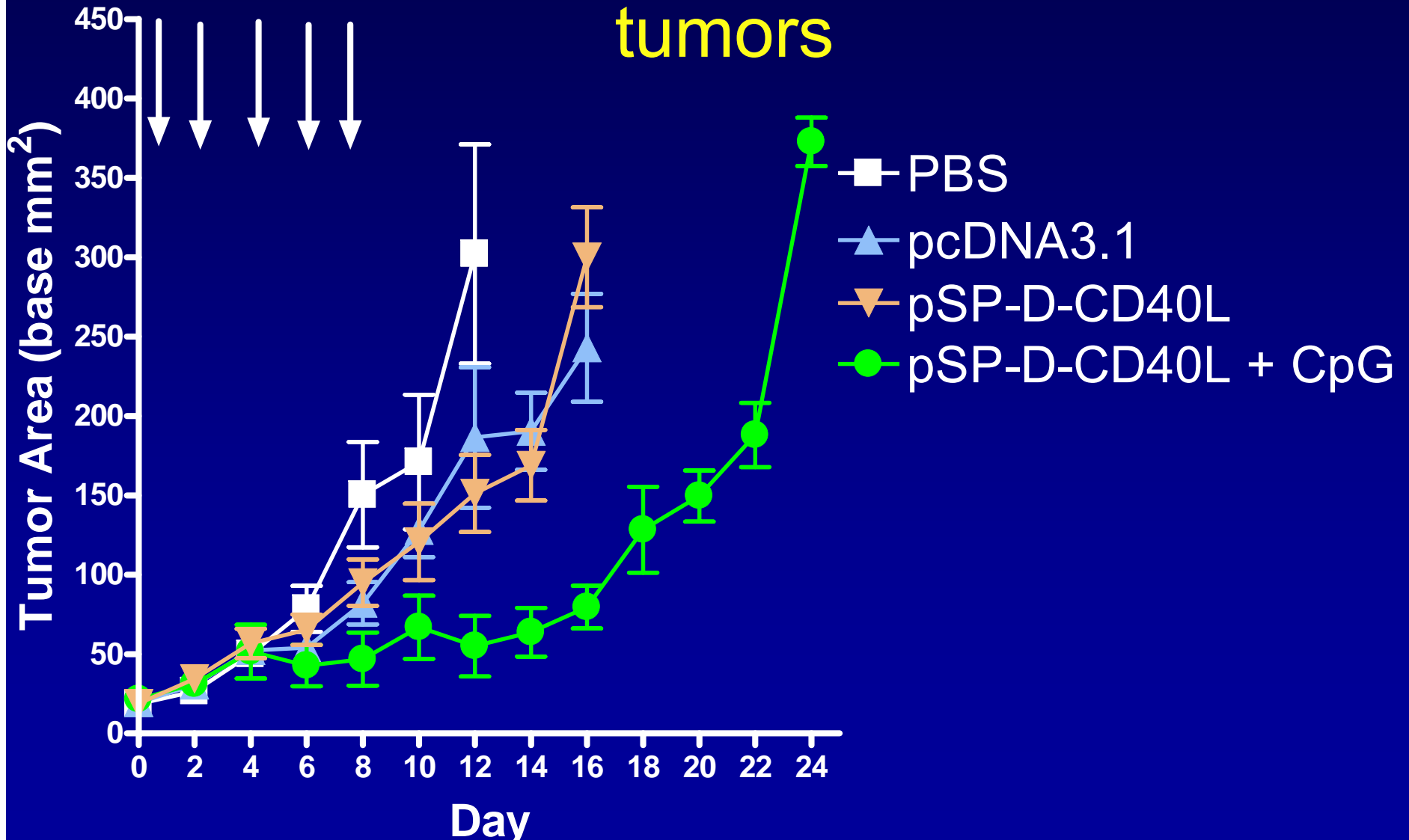
Synergy between CD40 activation and TLR agonists

- Lapointe et al (Hwu), *Eur J Immunol* 30:3291-3298, 2000.
 - ▶ enhanced DC activation to generate anti-melanoma CD8+ T cells in vitro
- Ahonen et al (Kedl and Noelle) *J Exp Med* 199:775-784, 2004.
 - ▶ enhanced generation of anti-OVA CD8+ T cells in vivo
- Napolitani et al (Lanzavecchia) *Nat Immunol* 6:769-776, 2005.
 - ▶ enhanced production of IL-12p70 and Th1 CD4+ T cells by human DCs in vitro

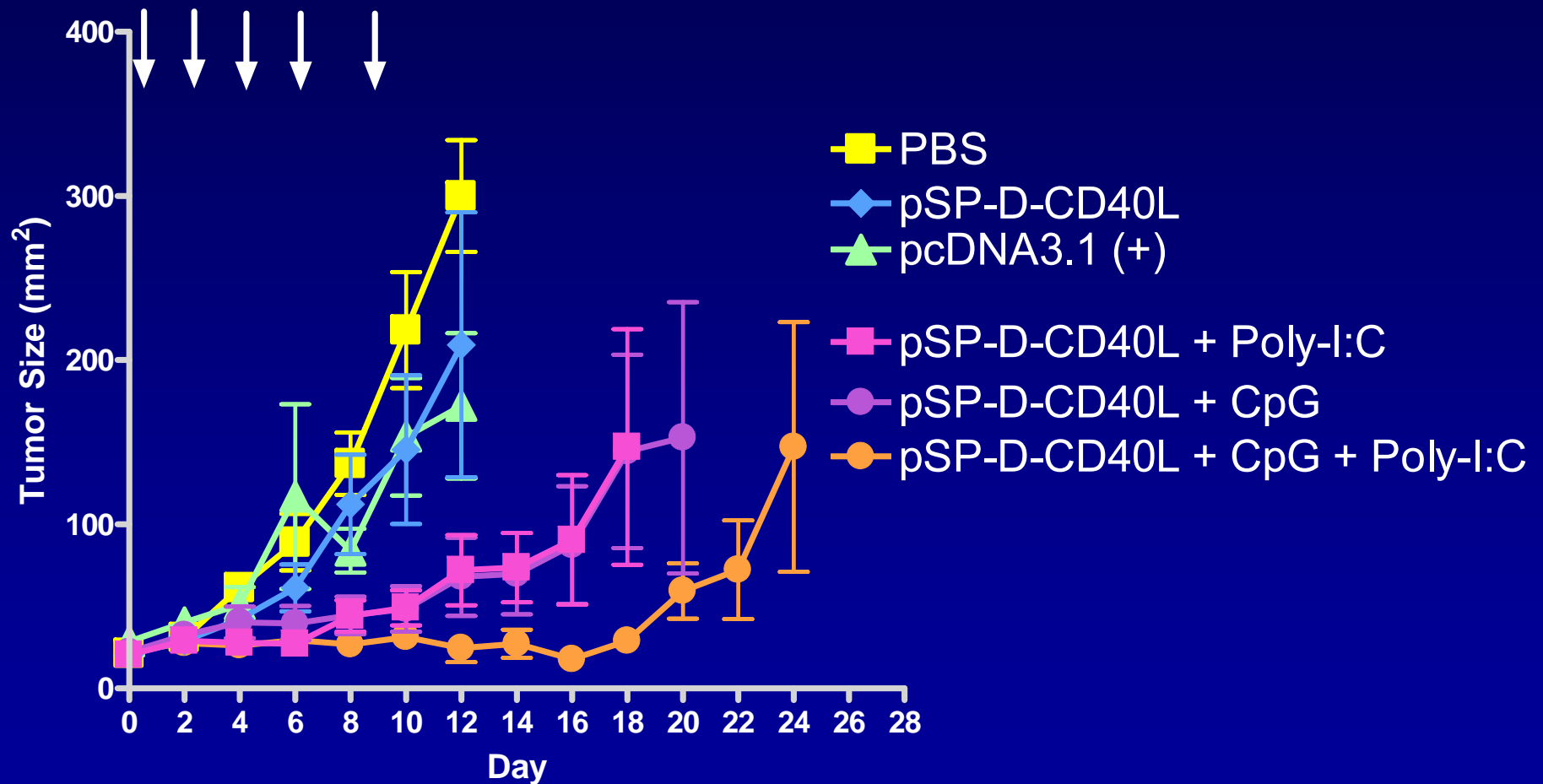
TLR and CD40L synergy against established B16-F10 tumors

| TLR | TLR Agonist | Effect |
|---------|-------------|--------|
| TLR 1/2 | Pam3CSK4 | - |
| TLR 2/6 | FSL1 | - |
| TLR 2/6 | MALP2 | - |
| TLR 3 | Poly I:C | + |
| TLR 4 | MPL | - |
| TLR 7/8 | Imiquimod | - |
| TLR 9 | CpG 1018 | +++ |

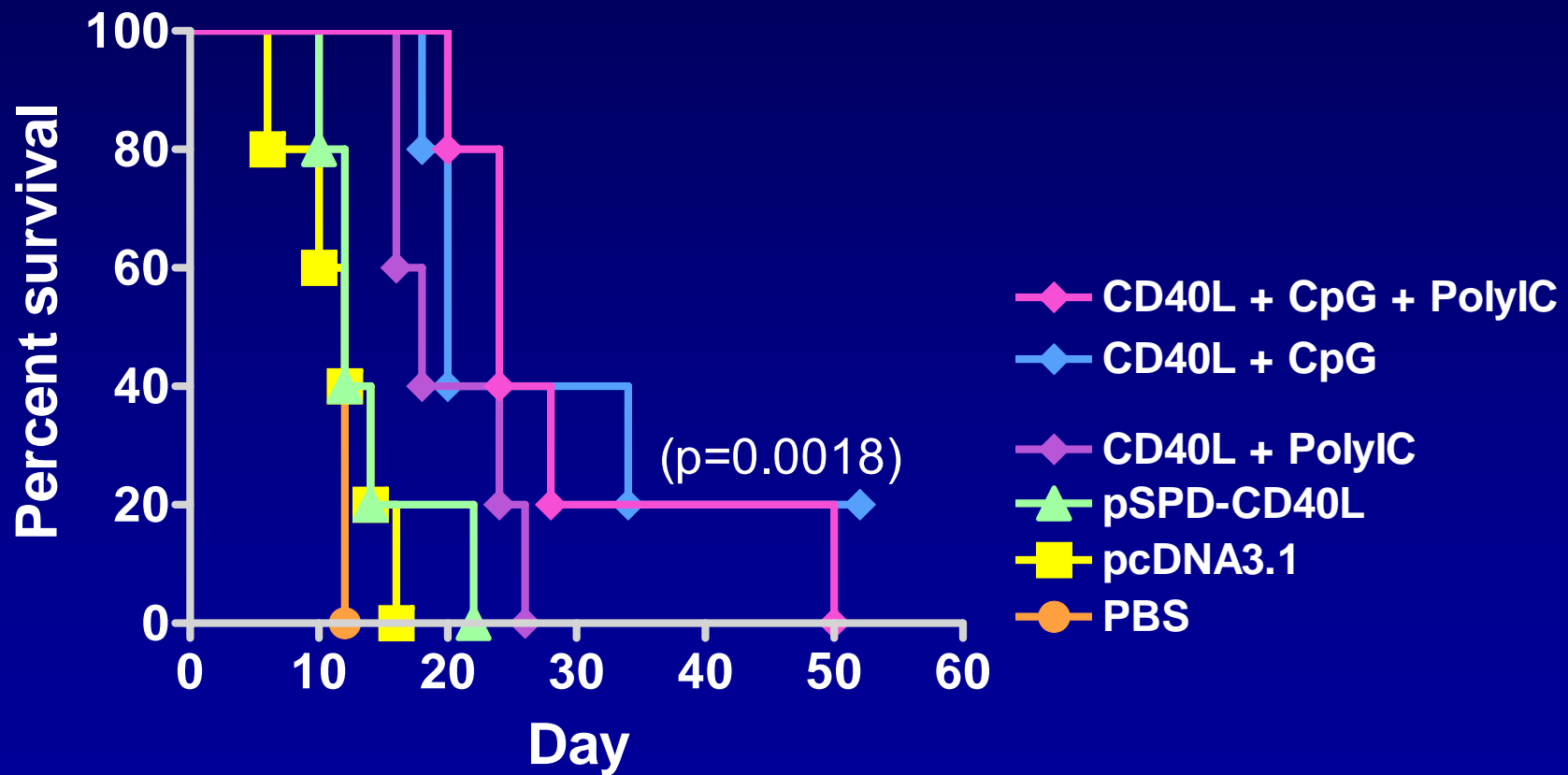
TLR9 stimulation (CpG) synergizes with CD40L against established B16-F10 tumors



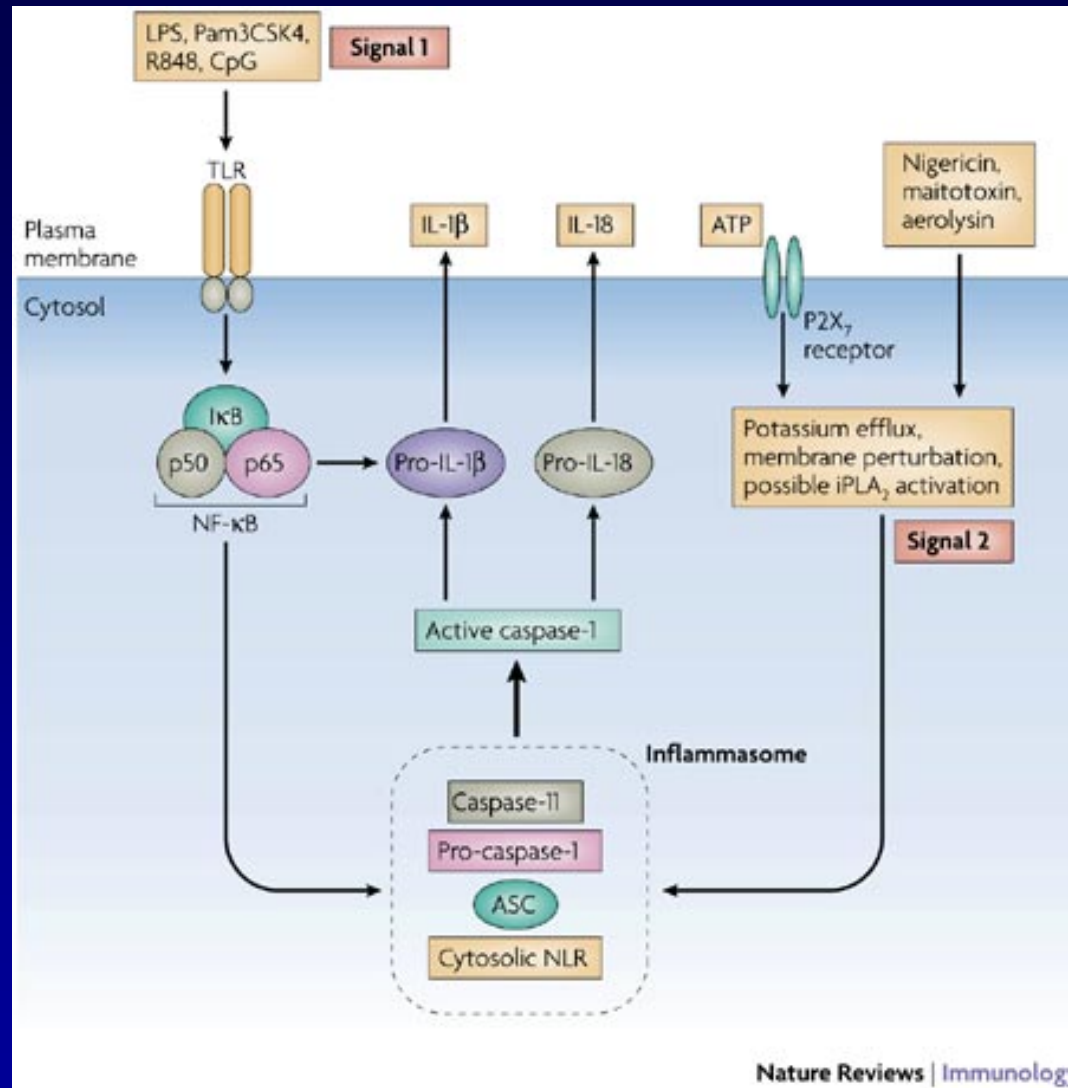
Combining Two TLR agonists with pSP-D-CD40L



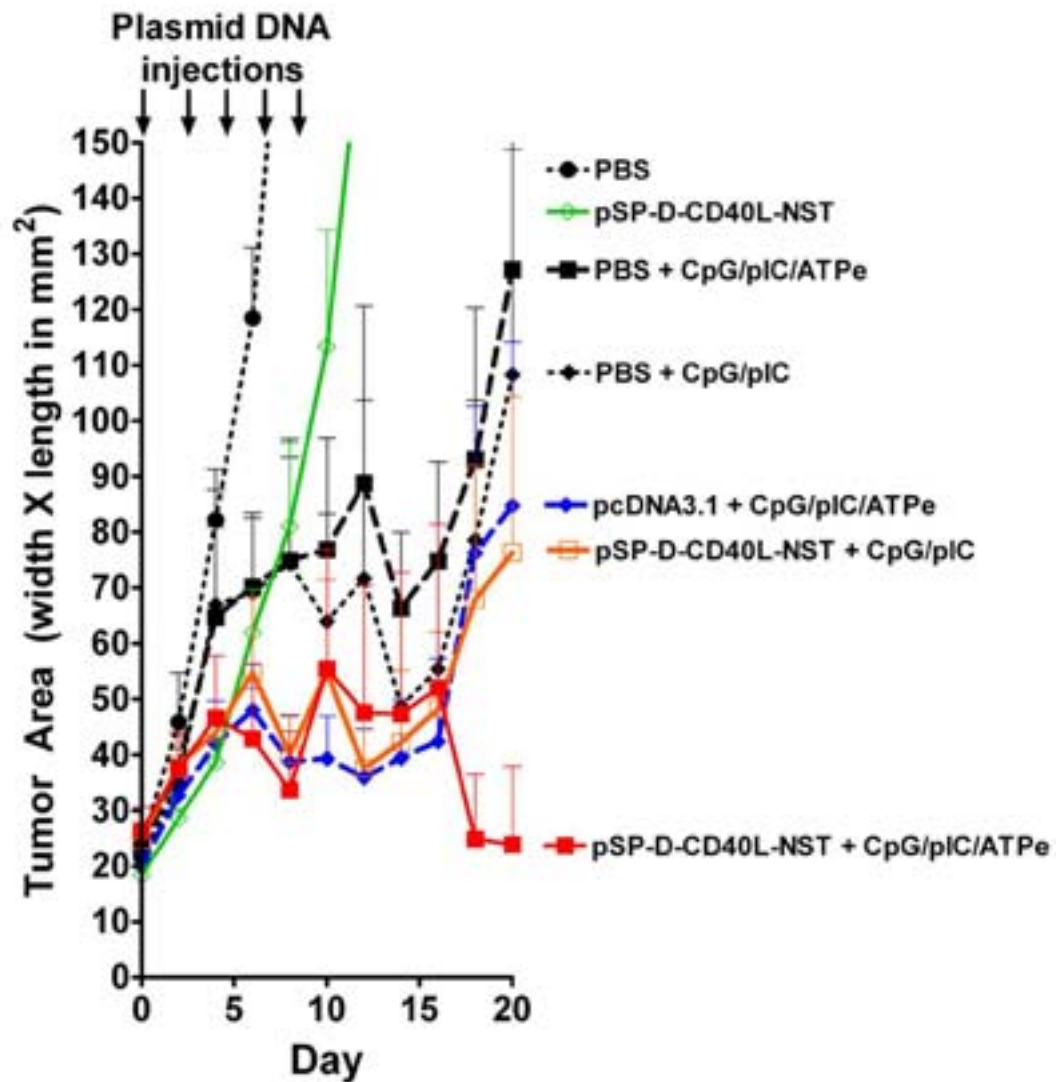
Combining Two TLR agonists with pSP-D-CD40L



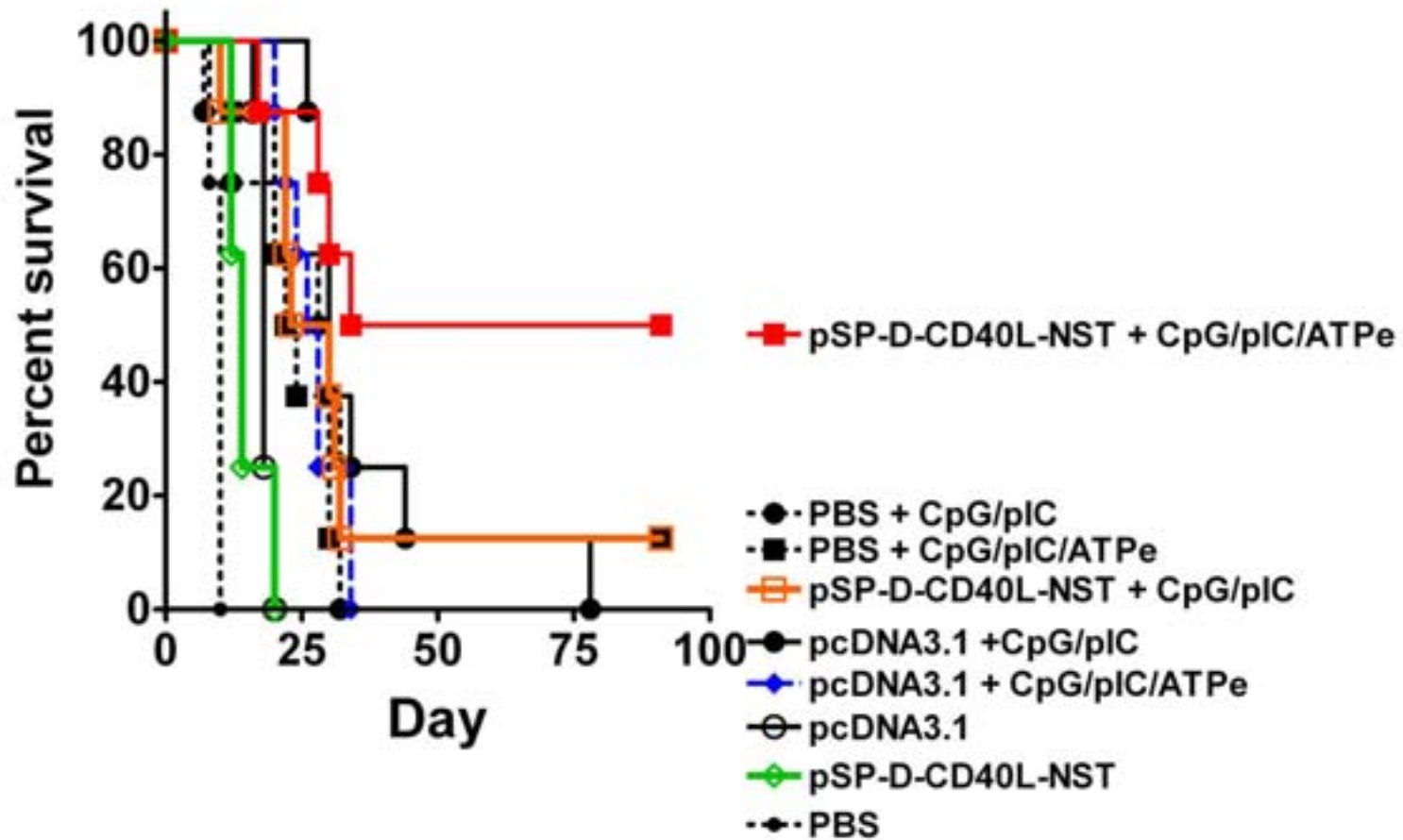
Inflammasome Activation of IL-1 β and IL-18



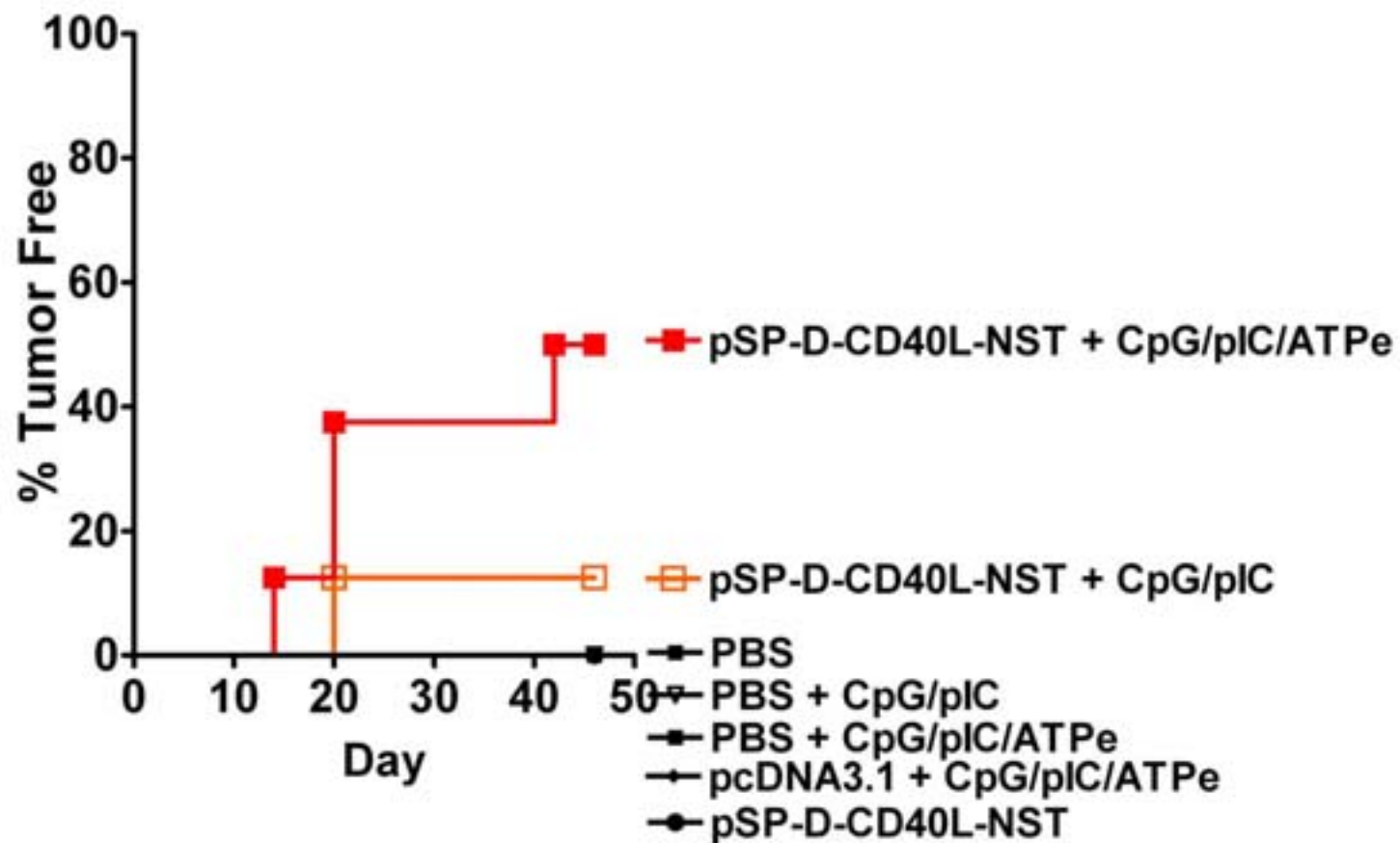
W200 B16F10 melanoma, effect of pSP-D-CD40L-NST plus CpG/poly(I:C) with or without ATPgammaS (ATPe)



W200 B16F10 melanoma, effect of combining ATPgammaS (ATPe) with pSP-D-CD40L-NST + CpG/poly(I:C)



W200 B16F10 Melanoma, Percent Tumor Free



Tumor Immunotherapy Conclusions

- For A20 lymphoma (Treg-rich), peritumoral injections of either GITRL or CD40L plasmids can cure established tumors
- For B16F10 melanoma (Treg-poor), peritumoral injections of GITRL are inactive, but CD40L plasmid + CpG + poly(I:C) + ATPe can cure established tumors

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