Immune therapy of cancer

"Aptamer-targeted RNA therapeutics"

Oligonucleotide-based cell-targeted immune stimulatory agents

Oligonucleotide aptamers

A novel platform technology for ligands with engineered specificity

Aptamer Library

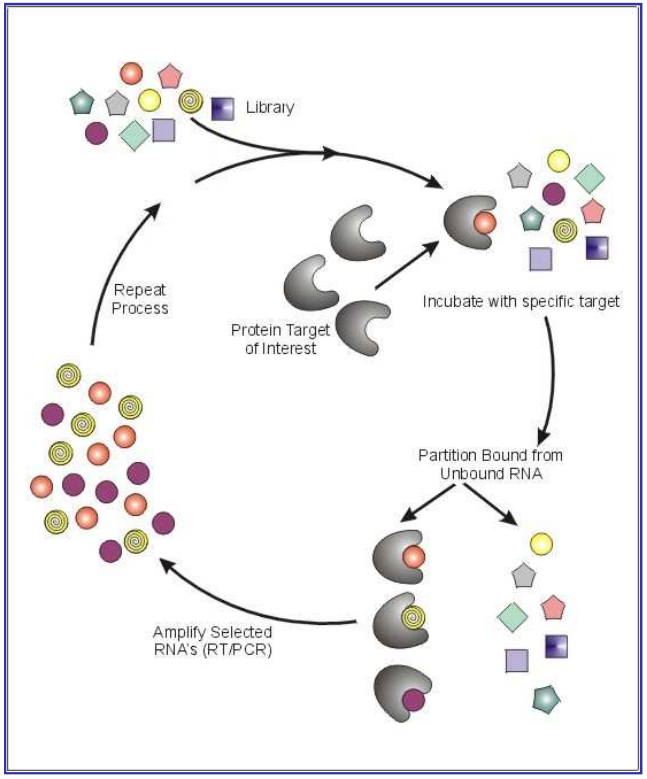
4^{40} possible sequences

AGGACGAUGCGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAGACGACUCGC

40 nucleotide random region

- Exhibit specificity and avidity comparable or exceeding that of antibodies
- Can be generated against most targets, proteins and small molecules

In vitro selection (SELEX)



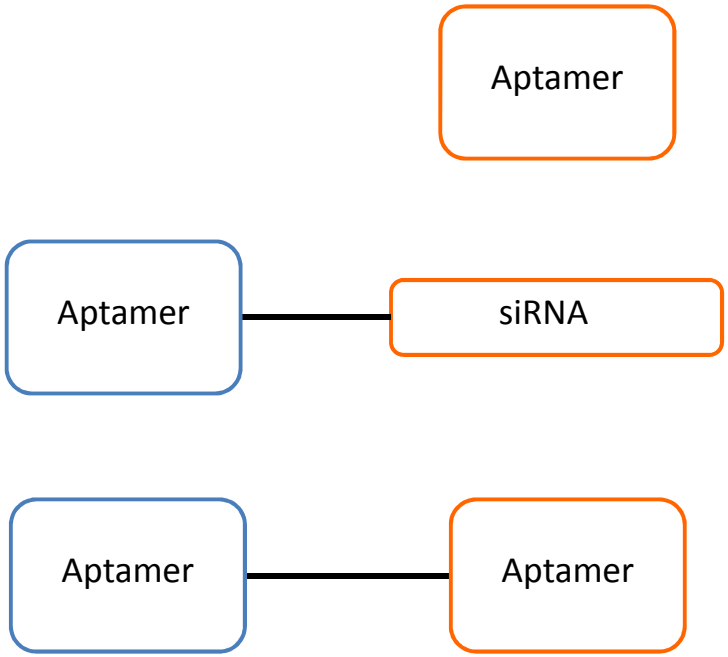
Custom-made ligands with engineered specificity

Antibodies versus aptamers

	Antibodies (Polypeptides)	Aptamers (Nucleic acids)
1. Synthesis • Development, manufacturing, regulatory process	Cell-based Costly & complex	Chemical (cell-free) Simple (by comparison)
2. Chemistry • Conjugation & modifications	Complex	Simple
3. Immunogenicity (repeated administrations)	Concern	Reduced to none
4. Cross-species ligands	Role of dice	A snap

Using aptamers to target nucleic acid-based immune modulatory agents to the tumor of the immune system

Targeting ligand **Therapeutic agent**



- CTLA-4
- 4-1BB
- OX40

Santulli-Marotto, Can. Res., 2007
McNamara, J. Clin. Invest., 2008
Dollins, Chem. Biol, 2008

- Tumor antigenicity
- T cell memory
- T cell resistance to TGFβ

Pastor, Nature, 2010
Berezhnoy, J. Clin. Invest, 2012
Brenemann, MS in preparation

- **Tumor costimulation**

Pastor, Mol. Ther., 2011
Schrand, Can. Immunol. Res., 2014

The NEW ENGLAND JOURNAL of MEDICINE

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Improved Survival with Ipilimumab in Patients with Metastatic Melanoma

F. Stephen Hodi, M.D., Steven J. O'Day, M.D., David F. McDermott, M.D., Robert W. Weber, M.D., Jeffrey A. Sosman, M.D., John B. Haanen, M.D., Rene Gonzalez, M.D., Caroline Robert, M.D., Ph.D., Dirk Schadendorf, M.D., Jessica C. Hassel, M.D., Wallace Akerley, M.D., Alfons J.M. van den Eertwegh, M.D., Ph.D., Jose Lutzky, M.D., Paul Lorigan, M.D., Julia M. Vaubel, M.D., Gerald P. Linette, M.D., Ph.D., David Hogg, M.D., Christian H. Ottensmeier, M.D., Ph.D., Celeste Lebbé, M.D., Christian Peschel, M.D., Ian Quirt, M.D., Joseph I. Clark, M.D., Jedd D. Wolchok, M.D., Ph.D., Jeffrey S. Weber, M.D., Ph.D., Jason Tian, Ph.D., Michael J. Yellin, M.D., Geoffrey M. Nichol, M.B., Ch.B., Axel Hoos, M.D., Ph.D., and Walter J. Urba, M.D., Ph.D.

Toxicity of systemically administered immune modulatory drugs in patients

- Anti-CTLA-4 mAb (Ipilumimab)

  Autoimmune toxicities, such as dermatitis, uveitis, colitis/enterocolitis, hepatitis and hypophysitis

- Anti-PD-1 mAb (Phase I trial)

  Mild inflammatory pathologies (one severe response)

- Anti-4-1BB mAb (Phase I/II trial)

  Grade 3+ neutropenia, elevated liver enzymes & severe hepatic toxicity

How to target immune stimulation to the disseminated tumors lesions of the cancer patient

…using a clinically feasible protocol

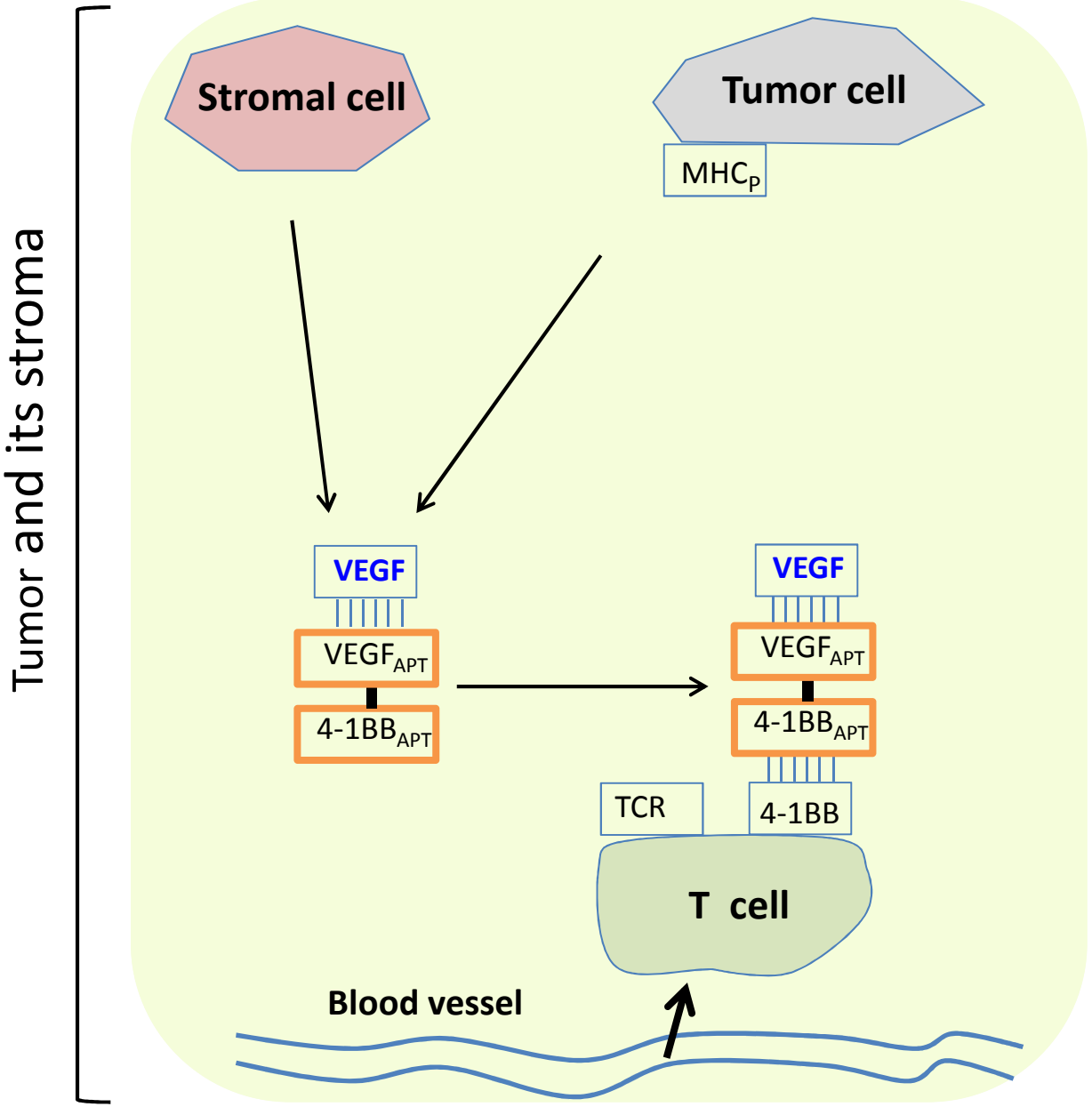
Disclosure Information

Eli Gilboa

All good ideas in my lab are coming from junior investigators

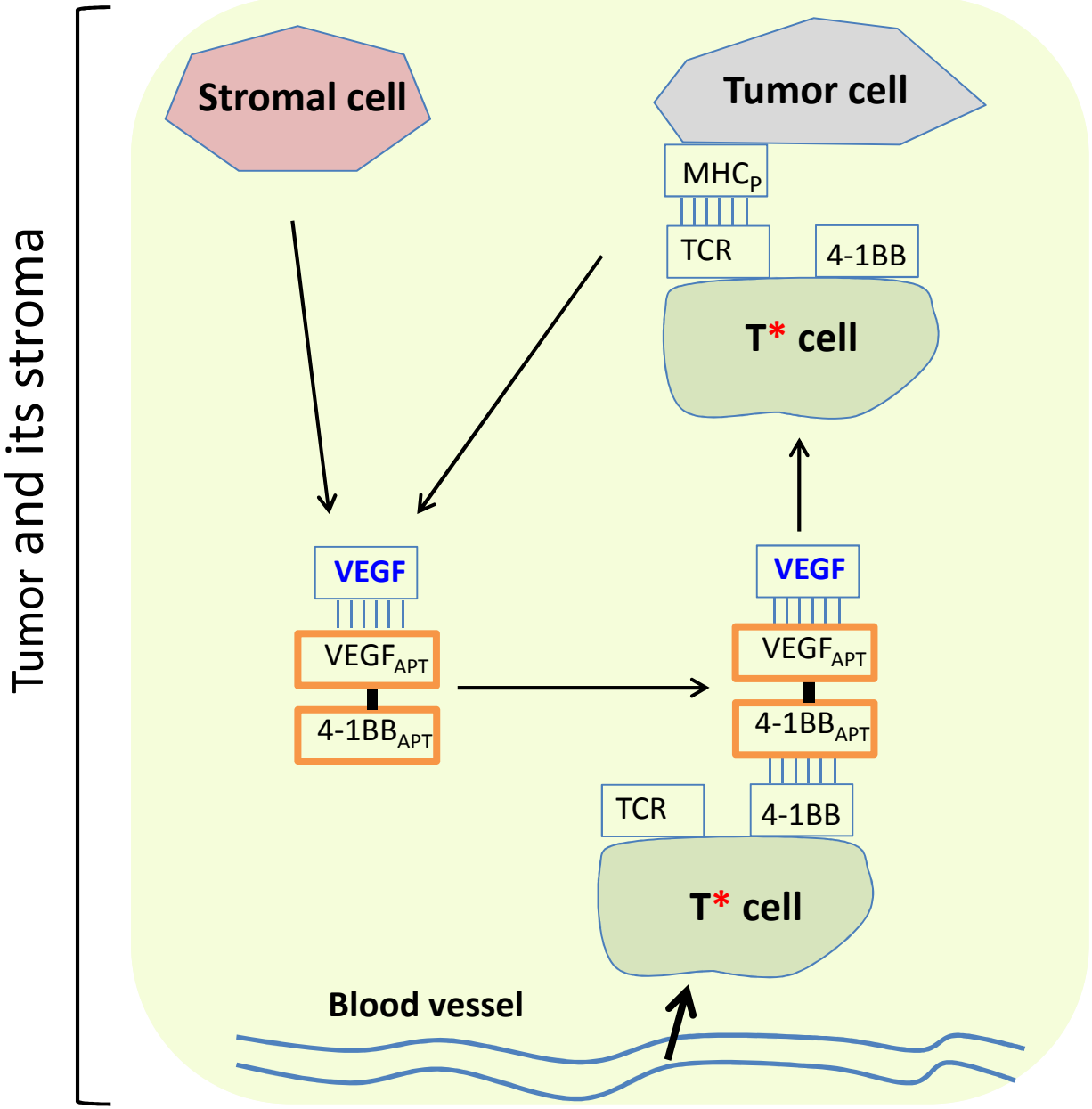
No other relationships to disclose

Targeting costimulation to the tumor stroma



Schrand et al. Can. Immunol. Res., 2014, 2:867

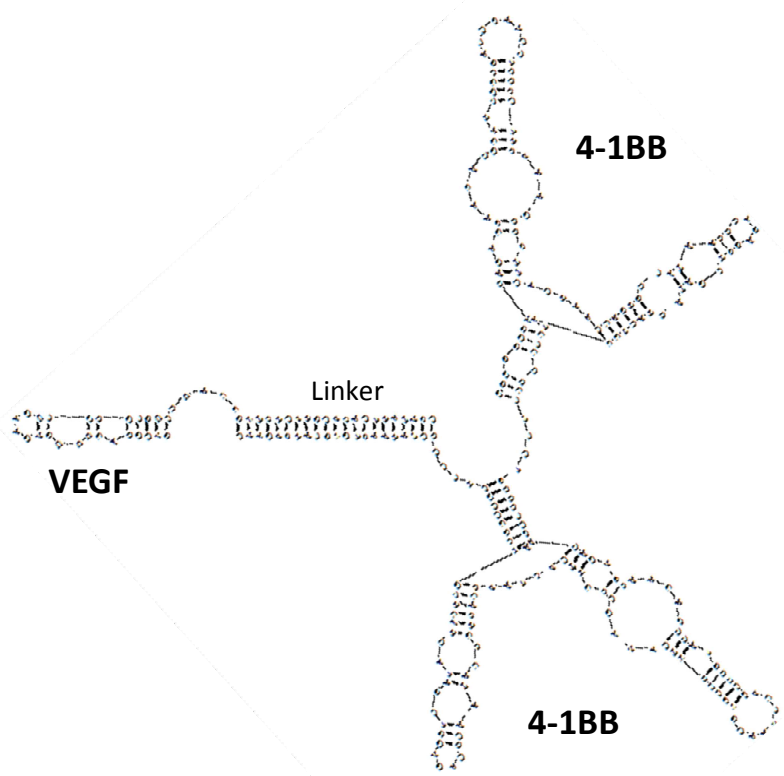
Targeting costimulation to the tumor stroma



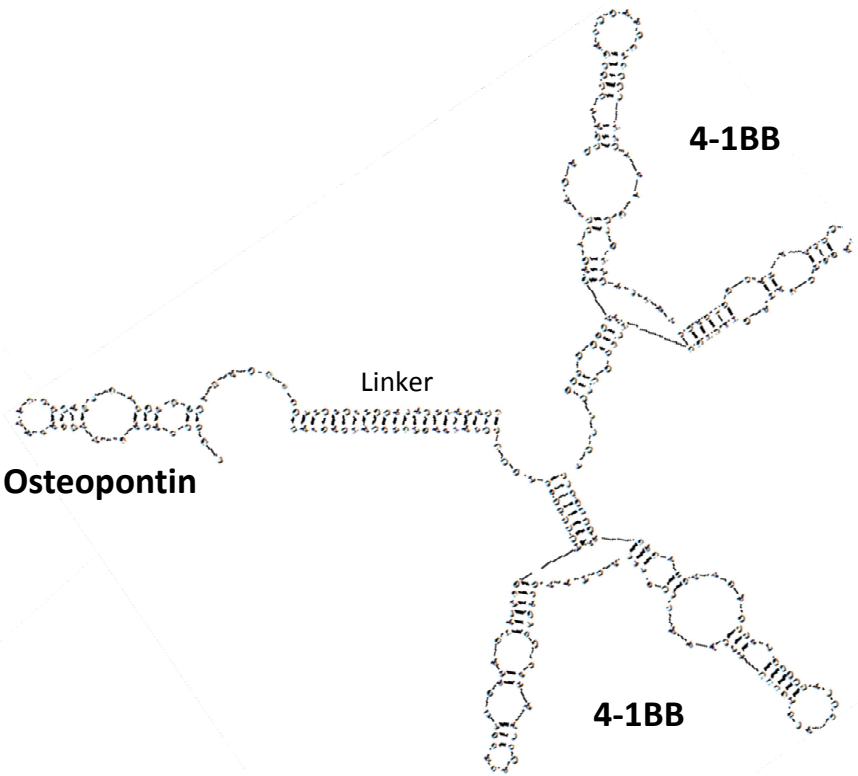
Schrand et al. Can. Immunol. Res., 2014, 2:867

VEGF and osteopontin (OPN) targeted dimeric 4-1BB heterotrimeric aptamer conjugates

VEGF-4-1BB



OPN-4-1BB



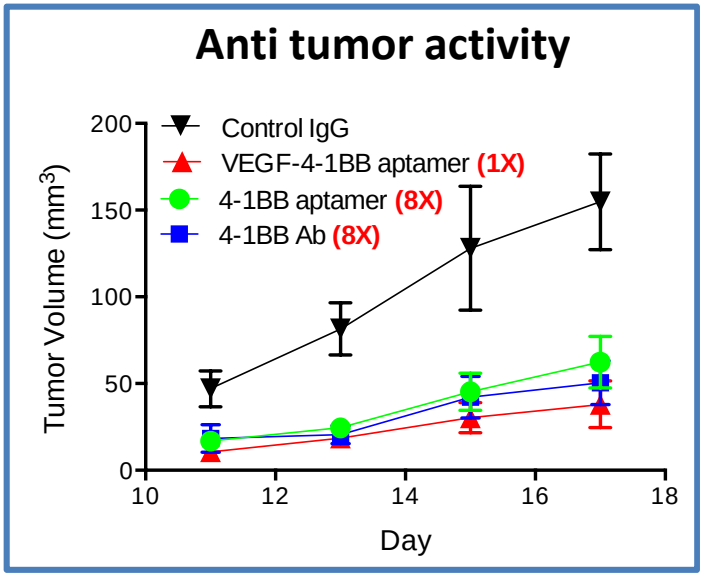
1. Whether inhibition of tumor growth is a result of targeting 4-1BB costimulation to the tumor via VEGF or osteopontin?

Schrand et al. Can. Immunol. Res., 2014, 2:867

2. Whether tumor stroma targeted 4-1BB costimulation will enhance the therapeutic index?

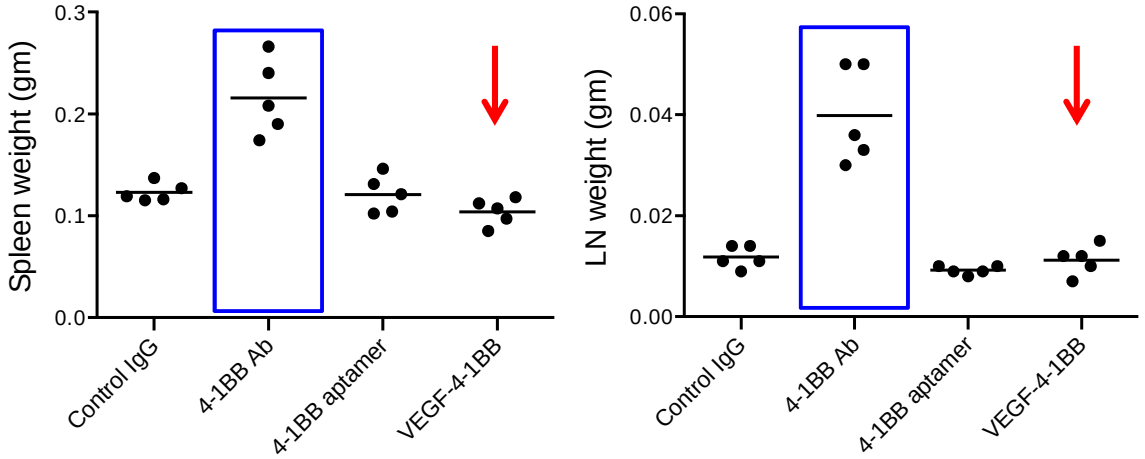
A. Reduces the effective dose for therapeutic benefit.
B. Reduce toxicity.

VEGF-4-1BB aptamer conjugate compared to 4-1BB antibody

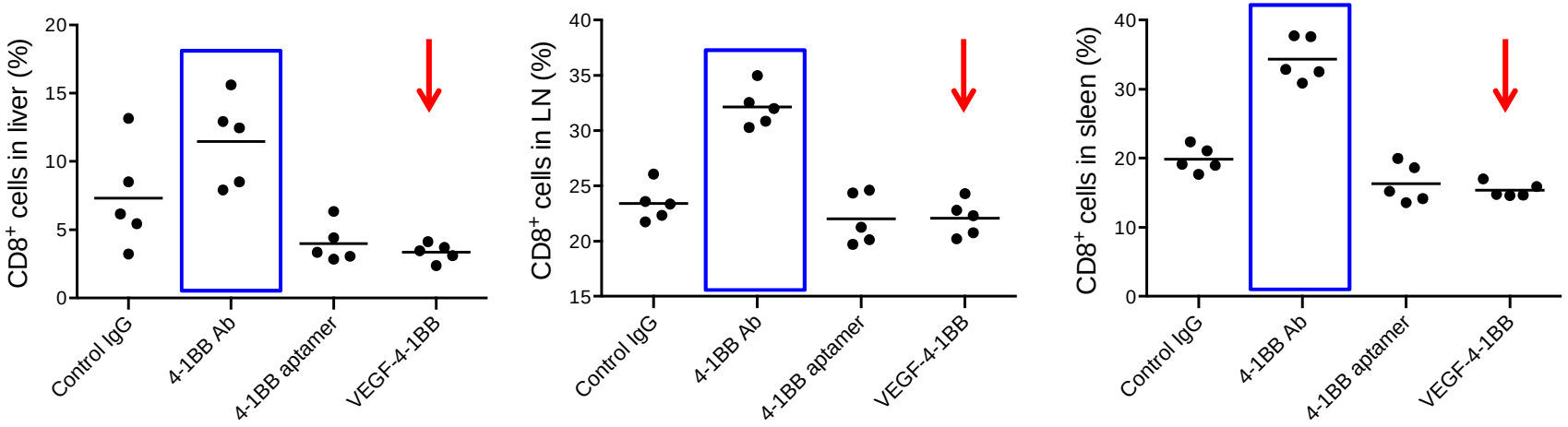


CD8+ T cell hyperplasia

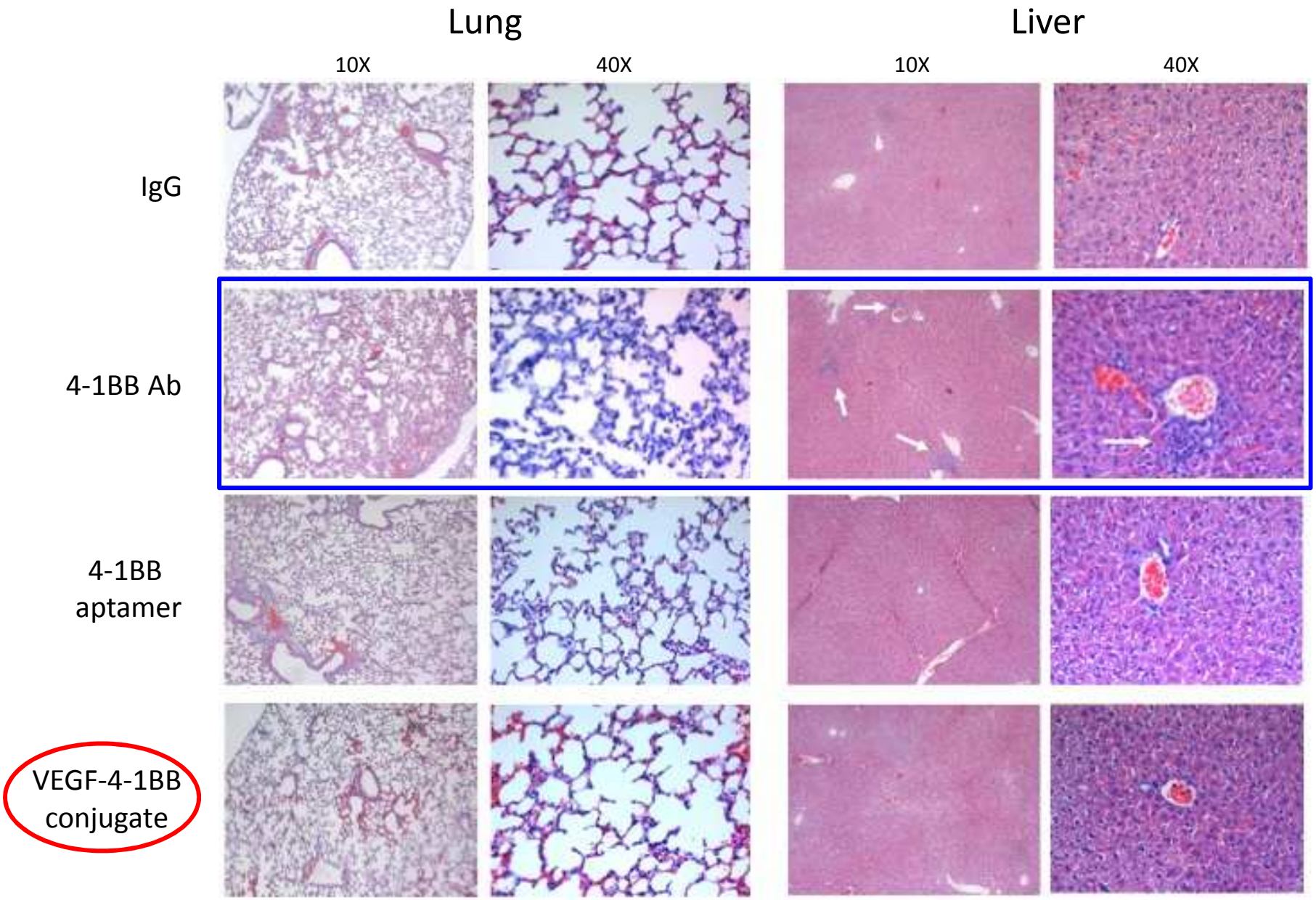
Organ cellularity



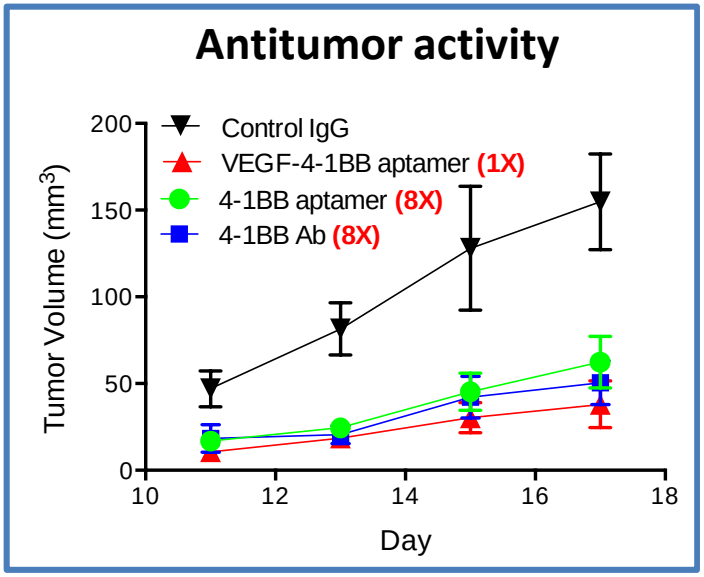
CD8⁺ T cells



VEGF-4-1BB aptamer conjugate compared to 4-1BB antibody



VEGF-4-1BB aptamer conjugate compared to 4-1BB antibody



CD8+ T cell hyperplasia

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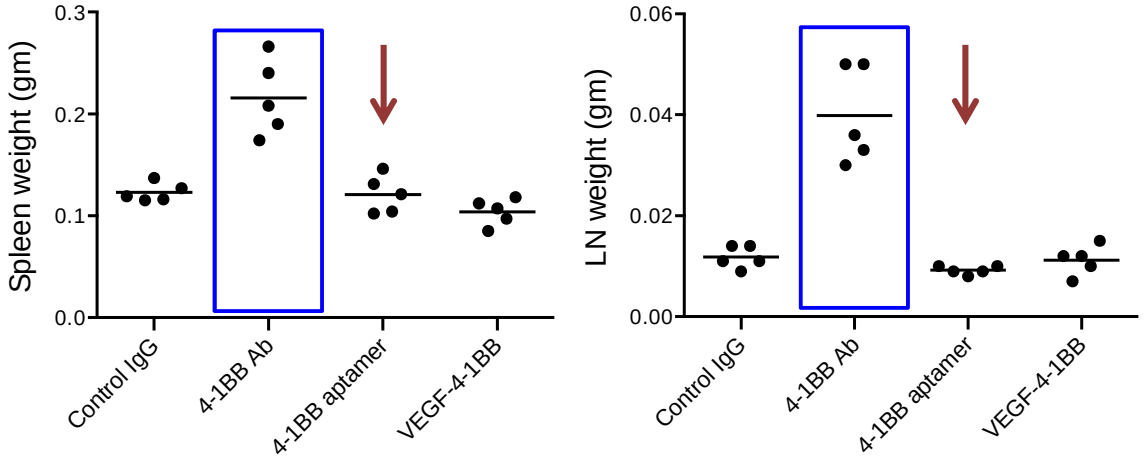

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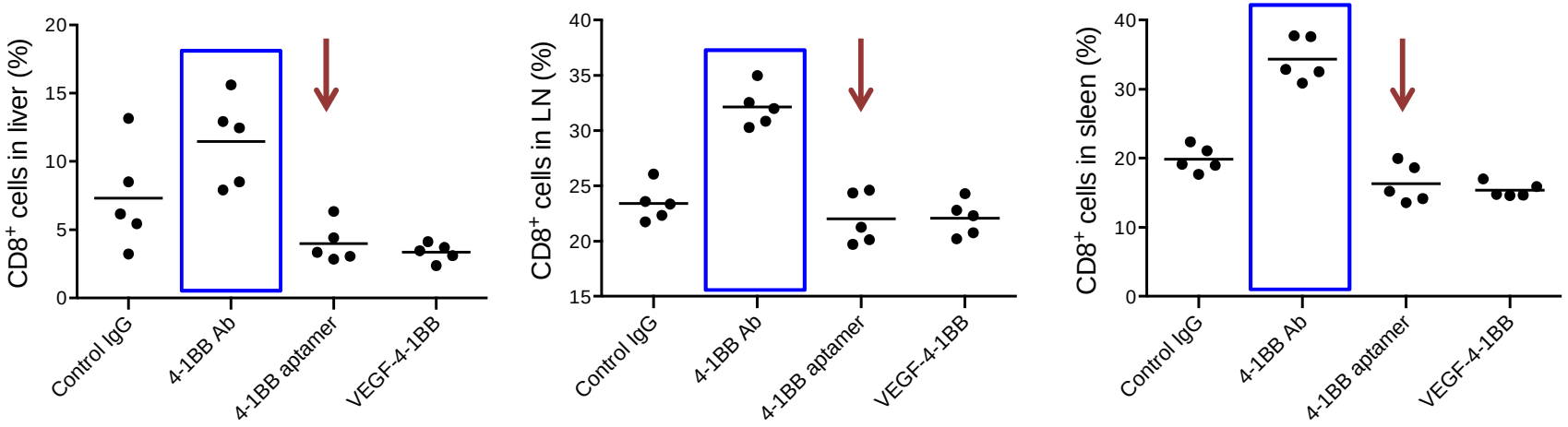
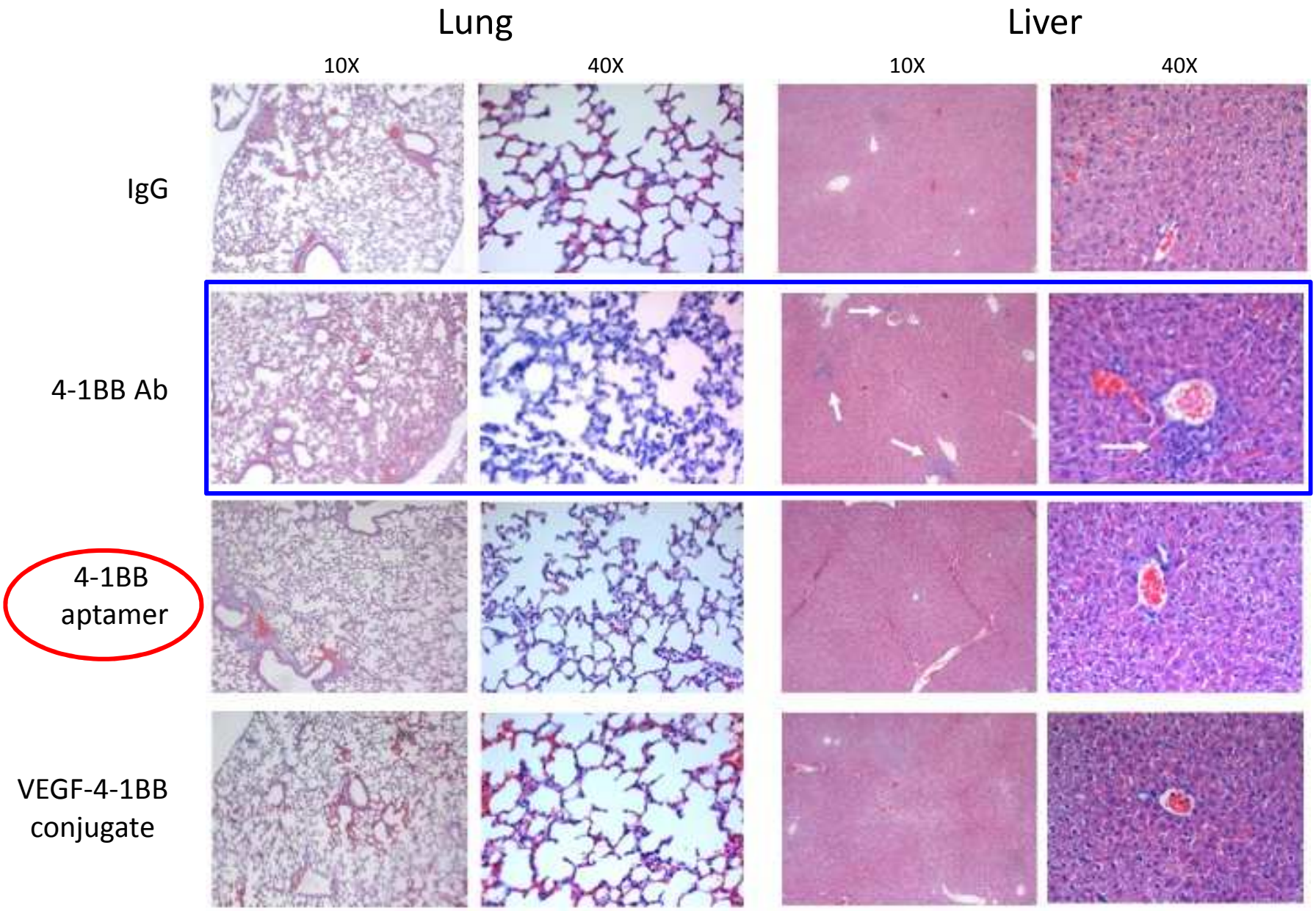


VEGF-4-1BB aptamer conjugate compared to 4-1BB antibody



Therapeutic index of 4-1BB costimulation

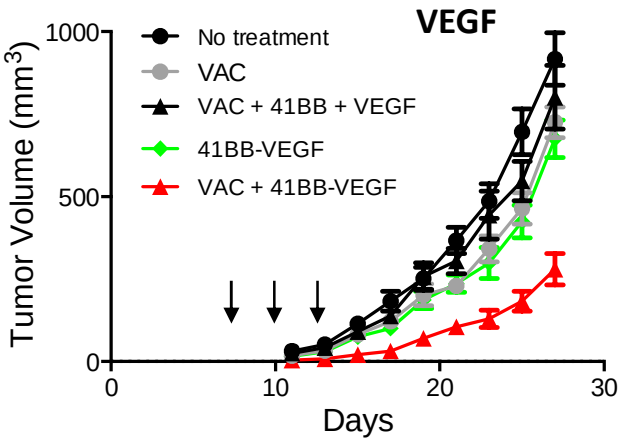
4-1BB aptamer > 4-1BB antibody

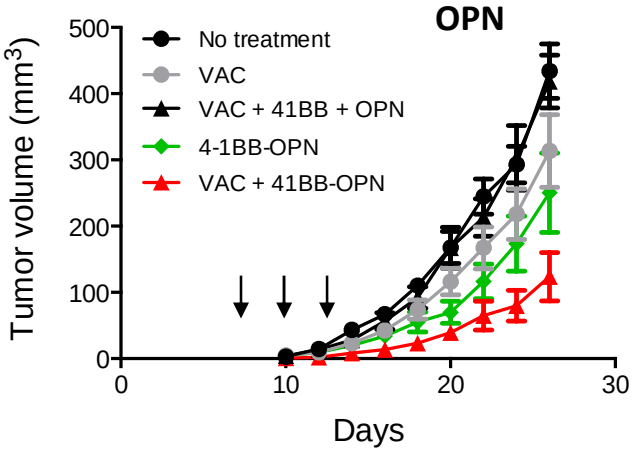
3. How potent is tumor stroma-targeted costimulation?

Targeting 4-1BB costimulation to tumor-secreted VEGF or osteopontin potentiates vaccine-induced tumor immunity

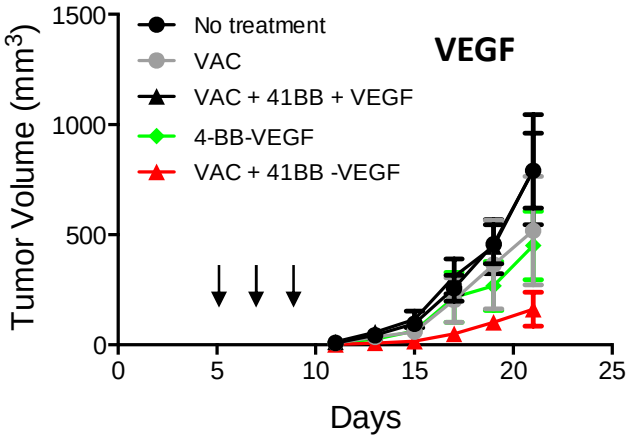
Therapeutic model: Tumor implantation → vaccination/treatment (days 7, 11,14)*

4T1 breast carcinoma (day 7)



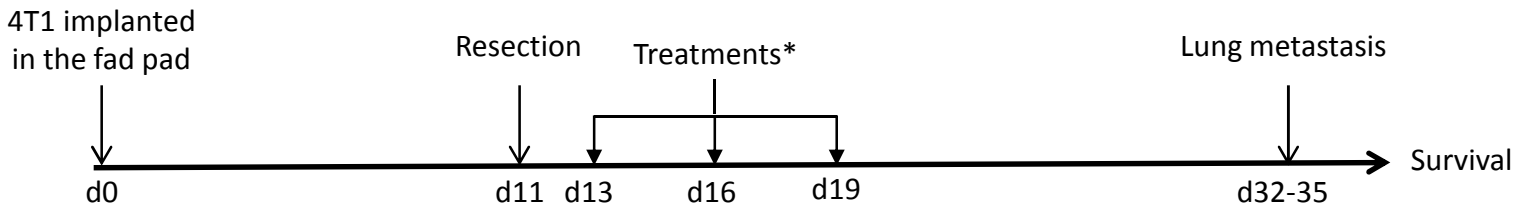


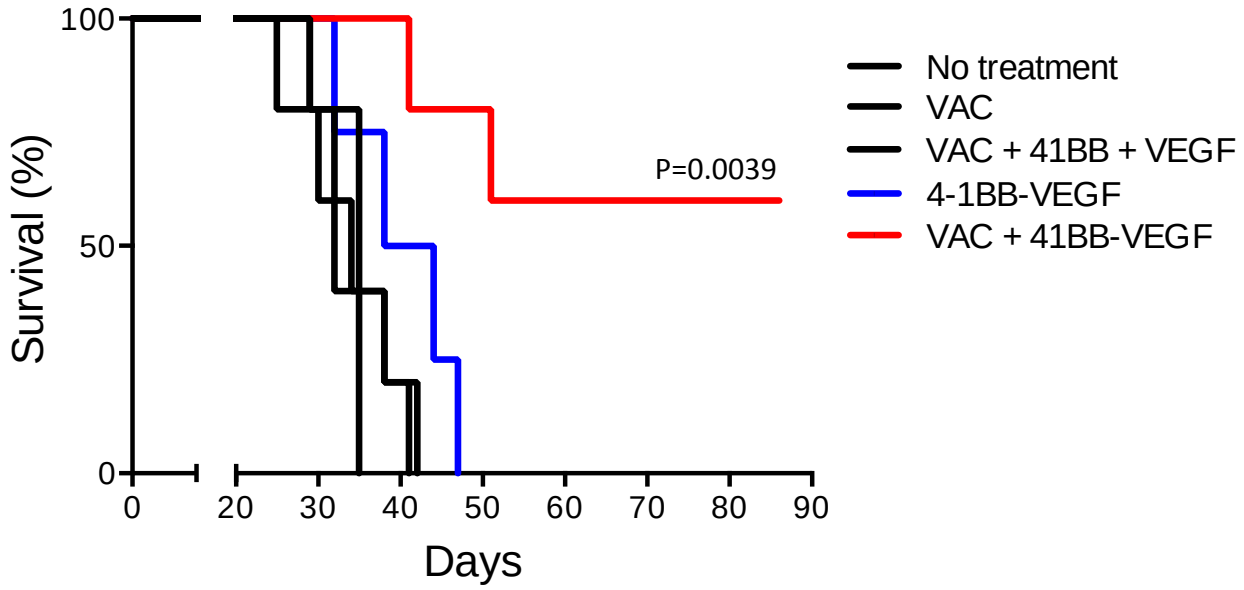
B16/F10 melanoma (day 5)



*Tumors implanted subcutaneously

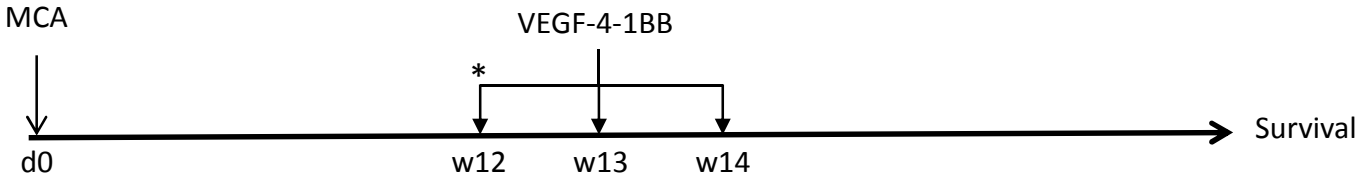
4T1 breast carcinoma post surgical metastasis model

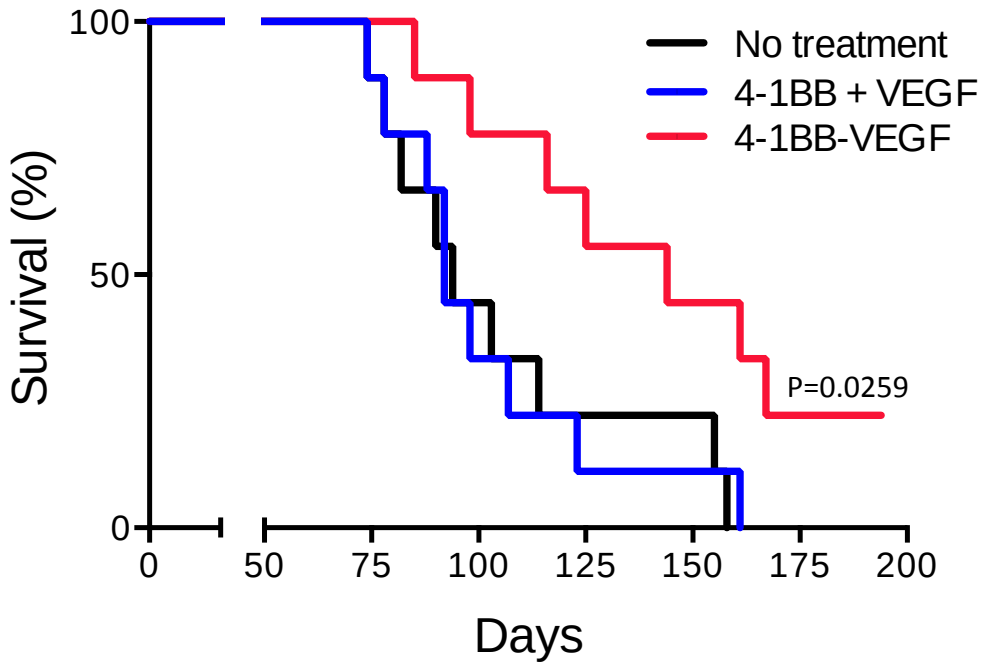




* When tumors become palpable: Vaccination → aptamer-siRNA conjugate

Methylcholantrene (MCA)-induced fibrosarcoma

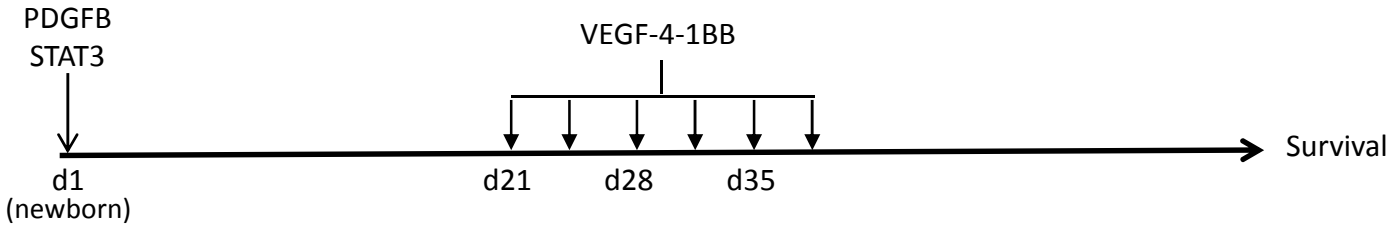


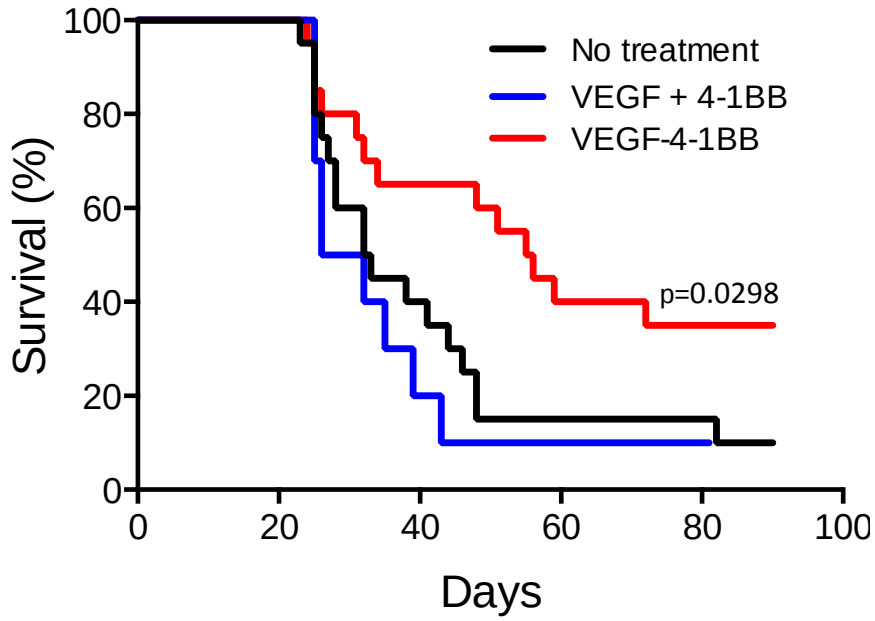


* When tumors become palpable.

Tumor-targeted 4-1BB costimulation enhancement of tumor immunity in an oncogene-induced autochthonous glioma model

Avian retroviral transduction of PDGF-B and STAT3 in nestin-driven ALV receptor transgenic mice*





*Wei et al., Can. Res., 2013, 73:3913

Stroma-targeted costimulation with bi-specific aptamers

- Potent
  - Increasingly relevant murine tumor model
  - First-generation conjugates
- Enhanced therapeutic index – reduced toxicity
- Broadly applicable
- Clinically feasible platform

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