

Oncolytic virus encoding CD47 blockade induces durable tumor regressions in murine models of lung cancer

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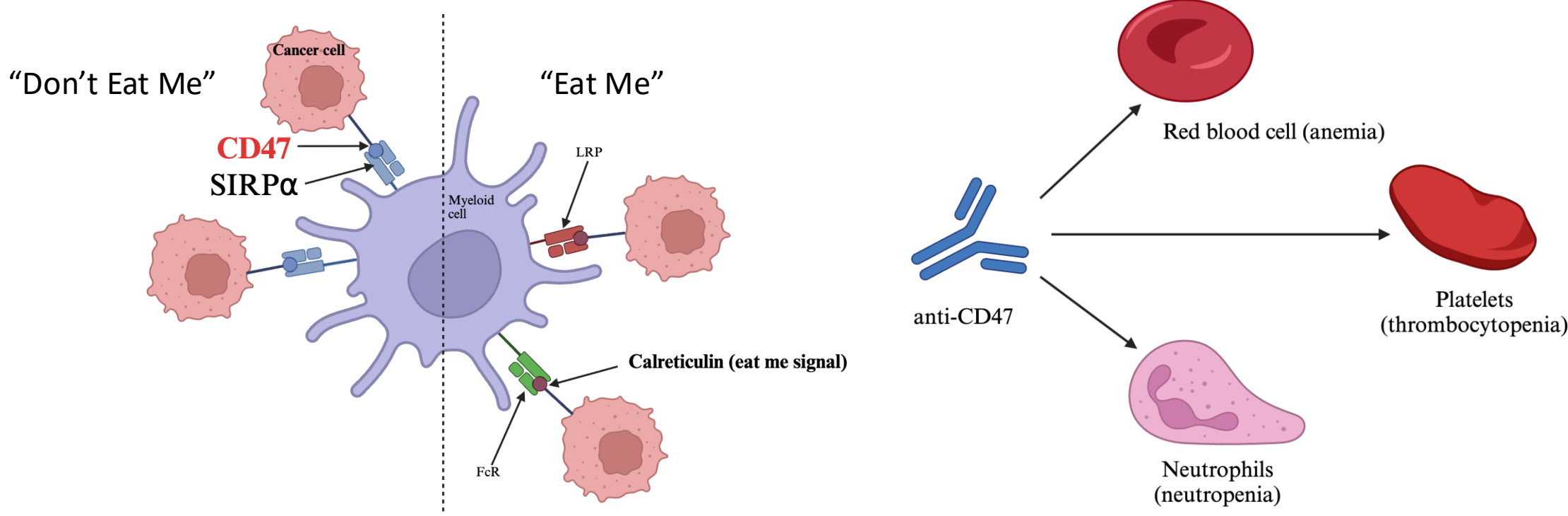
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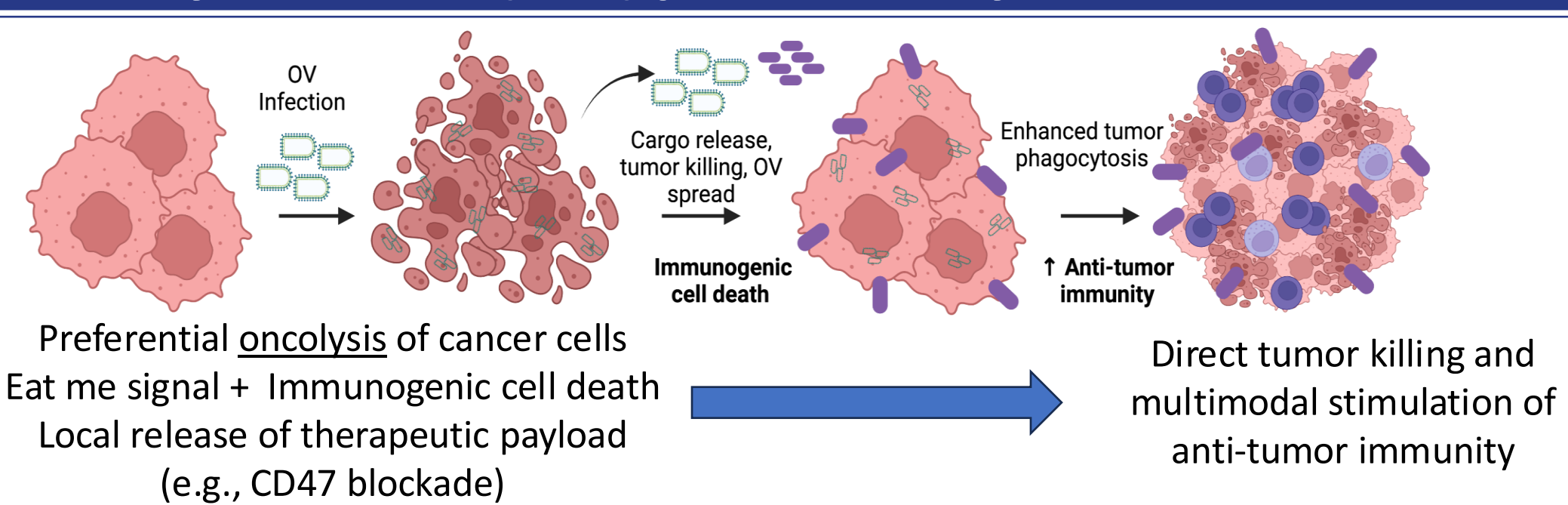
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Background: CD47 is promising therapeutic target in NSCLC

- CD47 is a cell surface protein upregulated in NSCLC (1)
- Binding of CD47 to SIRPα expressed on myeloid cells inhibits phagocytosis (1)
- CD47 blockade impairs lung tumor growth in preclinical studies (2)
- Efficacy of IV-administered CD47-targeted therapeutic antibodies has been limited by toxicities in patients that are attributable to the ubiquitous expression of CD47 on cells throughout the body (3-4)
- Alternative delivery strategies for CD47 blockade must be established to minimize systemic toxicity and maximize its therapeutic potential**



Oncolytic viruses (OVs) preferentially kill tumor cells



Study Rationale and Objective

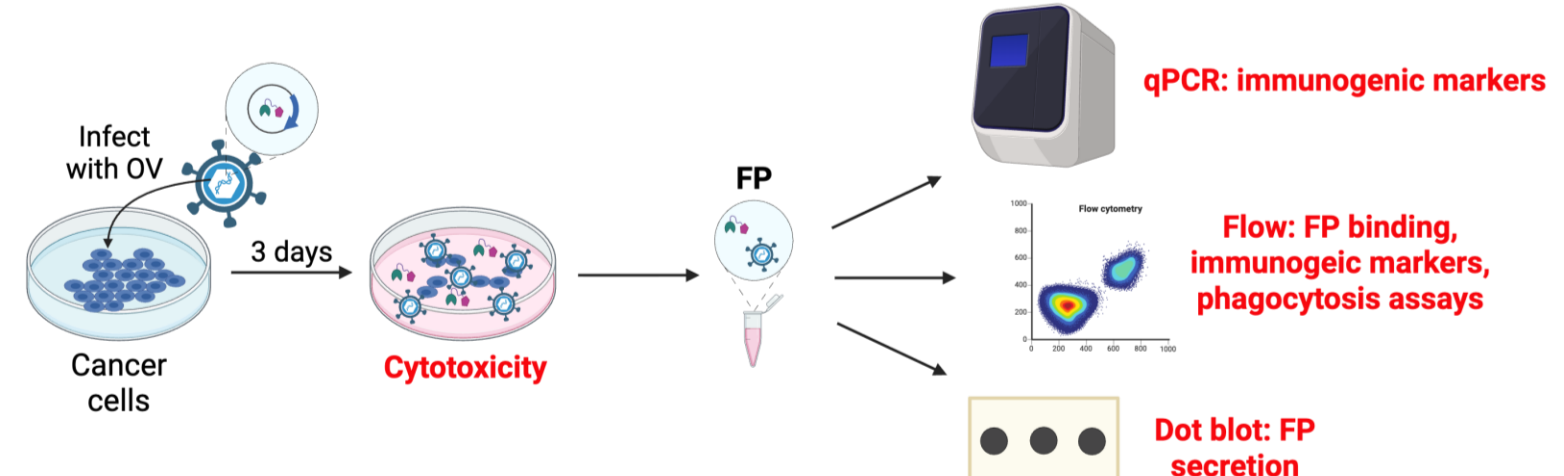
- Toxicities attributable to ubiquitous expression of CD47 on cells throughout the body has caused the failure of systemically administered CD47 therapeutic antibodies
- OVs could be leveraged to preferentially deliver CD47 blockade to tumor tissues *in vivo*, directly killing lung cancer cells and stimulating anti-tumor immunity to induce tumor regressions and minimize systemic toxicity.

Objective: Evaluate the tolerability and anti-tumor efficacy of a novel OV designed to deliver CD47 blockade for the treatment of NSCLC

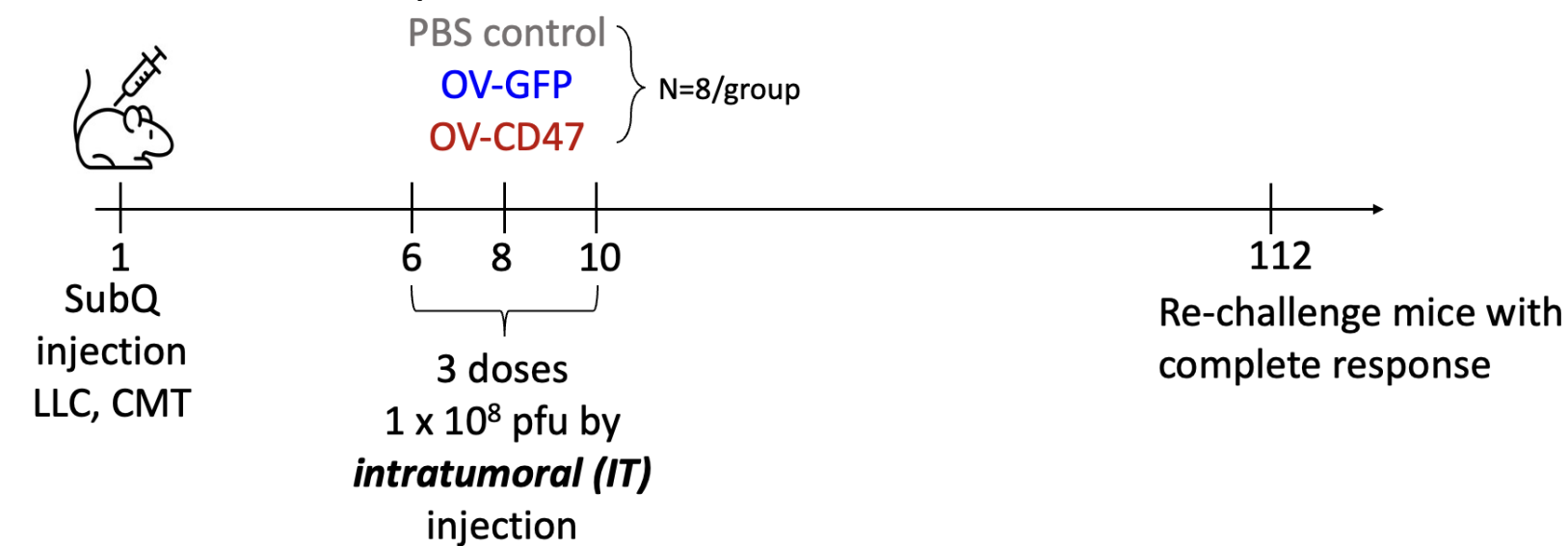
Hypothesis and Experimental Design

Hypothesis: Intratumoral delivery of OV-CD47, an OV encoding a fusion protein (FP) that blocks CD47 function, will be well tolerated and potentiate the anti-tumor efficacy of CD47-targeted immunotherapy in syngeneic lung tumor models

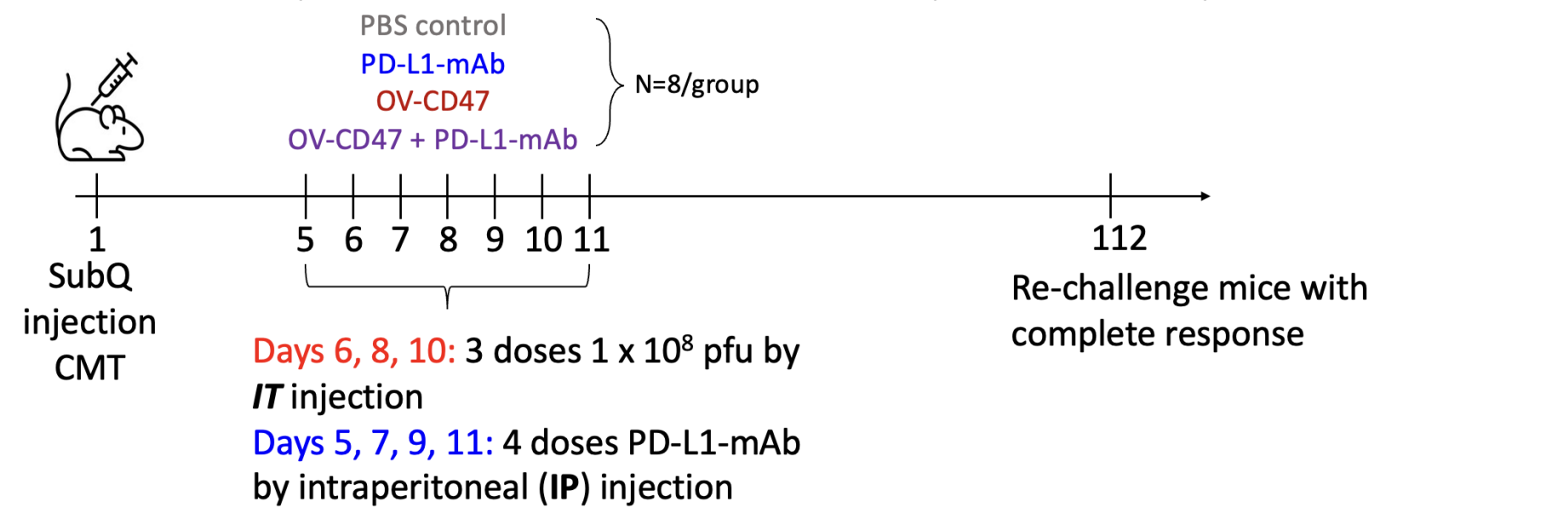
Aim 1: Validate OV-CD47 mechanism of action with proof-of-concept *in vitro* experiments



Aim 2: Assess OV-CD47 efficacy in mouse models of NSCLC and characterize its immunological effects



Aim 3: Evaluate the efficacy of OV-CD47 and anti-PD-L1 therapeutic antibody combination therapy



RESULTS: OV-CD47 infects and kills cancer cells, increases immunogenicity, and directs synthesis and secretion of a fusion protein that binds to cancer cells and enhances phagocytosis

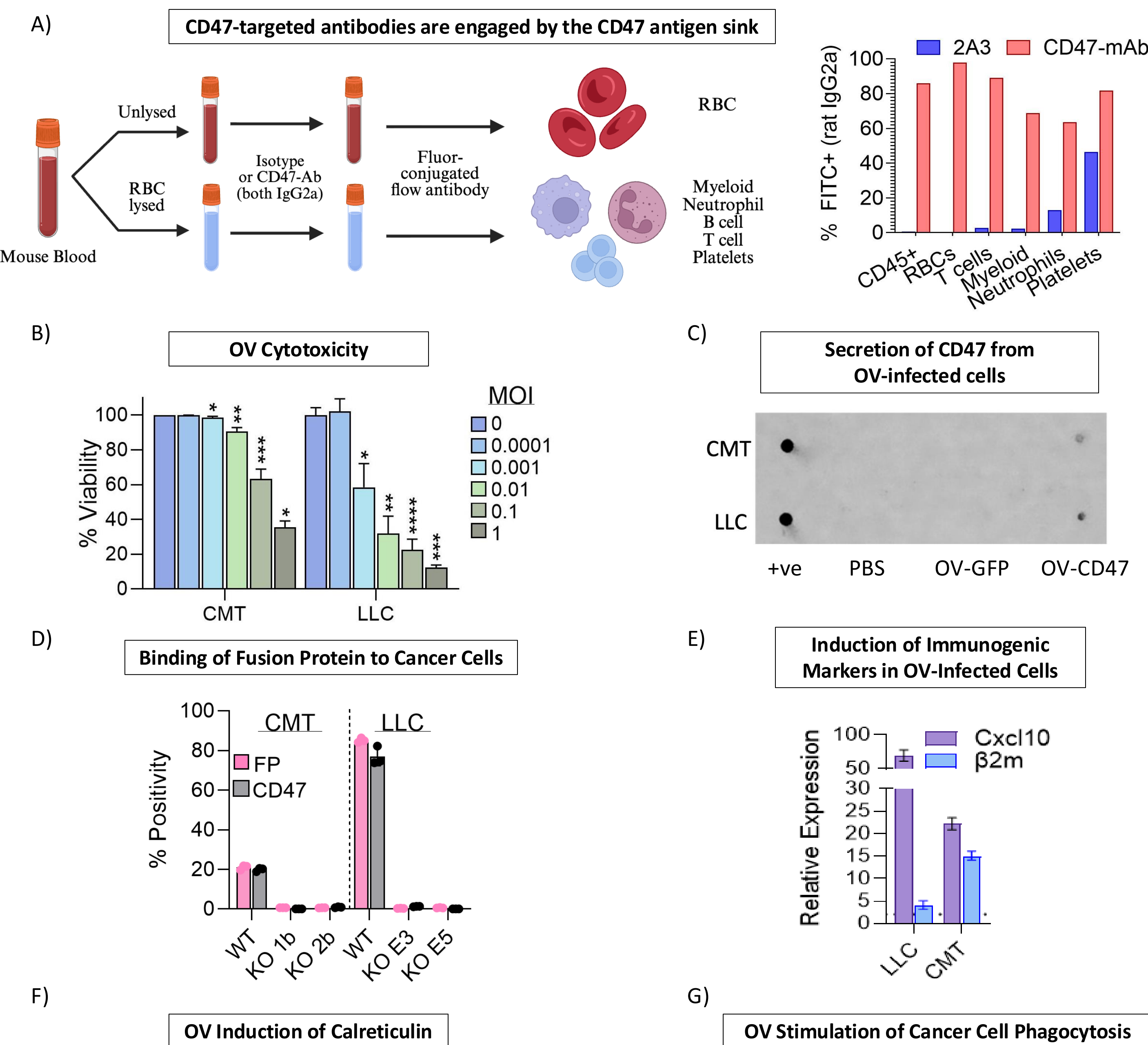


Figure 1. *In vitro* proof-of-concept studies demonstrating binding of CD47 to CD47⁺ cancer cells and the immunogenicity of OV-CD47. (A) CD47-mAb binding to cells in mouse blood after treatment with CD47-mAb/2A3 (both IgG2a). Binding was detected using a FITC conjugated anti-IgG2a. (B) Cytotoxicity of OV-GFP 48h after infection. MOI = multiplicity of infection. Viability was assessed with crystal violet staining and normalized to uninfected controls (MOI=0). (C) Dot blot showing secretion of CD47 FP in supernatants collected from OV-CD47 infected cells. (D) Flow cytometry detection of CD47 FP binding to WT vs. CD47 knockout cancer cells. (E) qPCR for *Cxcl10* and *β2m* in cells surviving after 48hr infection with OV-CD47 at MOI=0.01. Dotted line indicates a 2-fold expression change. (F) Flow cytometry detection of calreticulin surface expression on cancer cells surviving after 48hr treatment with PBS or OV-CD47 at MOI=0.01. (G) Phagocytosis assays done by co-culturing mCherry (mCh)⁺ LLC or CMT cells with LPS- and IFNγ-stimulated BMDM for 4h. Phagocytosed cells were calculated as % mCh⁺CD80⁺ cells over total live cells.

Conclusions and Future Directions

Conclusions:

- OV-CD47 infects and kills lung cancer (LC) cells and enhances their phagocytosis by macrophages.
- OV-CD47 induces strong anti-tumor activity in mice and is well tolerated.
- Re-challenge experiments suggest that OV-CD47 can confer long-term immunologic memory against LC cells.
- Upregulation of PD-L1 is a potential mechanism of resistance to tumor immunity induced by OV-CD47.
- Combining OV-CD47 with PD-L1 blockade is well tolerated and enhances anti-tumor activity and response durability.

Future directions:

- Perform immune profiling experiments to characterize immune responses induced by OV-CD47 treatment.
- Test the efficacy of human-specific OV-CD47 in human models of lung cancer.

RESULTS: OV-CD47 is well-tolerated, significantly delays tumor growth, and eradicates tumors in some CMT and LLC tumor-bearing mice

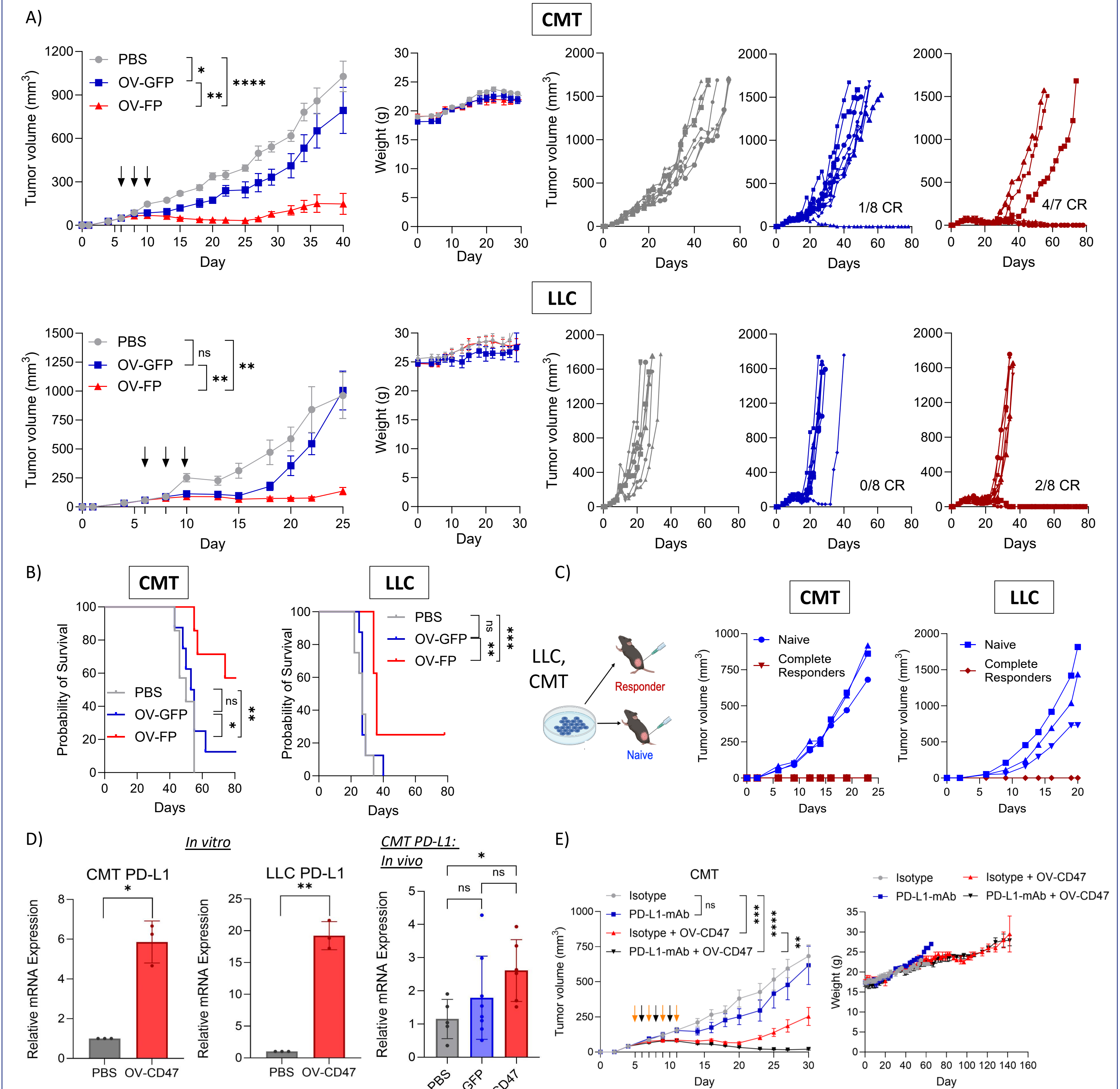


Figure 2. *In vivo* efficacy studies with CMT and LLC tumor-bearing mice. A) Efficacy and tolerability of OV-CD47 in CMT and LLC subq models using 3 doses of 1x10⁸ pfu (black arrows). Average tumor volumes and individual growth curves are shown with the number of complete responders (CR). Increases in body weight over the study indicates OV are well-tolerated. Error bars: mean±SEM. Asterisks show significance for tumor size comparisons on Day 25/40 (two-tailed t-test). B) Survival curves for OV treatment in the CMT and LLC models. Asterisks indicate significance for log-rank tests. C) Re-challenge experiment indicating long-term tumor immunity induced by OV-CD47 in cured mice. Subq injected CMT or LLC cells formed tumors in age-matched, tumor and treatment naïve controls (blue curves) but not in previously treated complete responder mice (N=4 for CMT, N=1 for LLC; red curves). D) *Pd-L1* mRNA upregulation in both OV-treated CMT and LLC cells *in vitro*, as well as *in vivo* endpoint tumors in the CMT tumor model. Significance was assessed using a one-sample t-test. E) OV-CD47 and PD-L1-mAb combination therapy showing strongest anti-tumor effects in the dual OV-CD47 and PD-L1-mAb treatment group, as well as good treatment tolerability in all groups. Error bars in tumor growth curves indicate mean ± SEM. Survival was analyzed using Mantel Cox log-rank tests. Tumor size comparisons between treatments were assessed using a two-tailed student's t-test.

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