Development of MEM-288, a dual-transgene armed and conditionally replication-enhanced oncolytic adenovirus with potent systemic antitumor immunity

MOFFITT CANCER CENTER

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Introduction

The "immune-desert" phenotype exhibits the virtual absence of T cells in tumor beds, possibly resulting from immune ignorance and/or lack of priming. Patients with such tumors rarely respond to immune checkpoint inhibitors (ICI) indicating that new strategies are required to increase ICI response against such tumors. Oncolytic Viruses (OVs) are unique for their ability to specifically replicate in cancer cells[1]. Recent studies indicate that stimulation of host anti-tumor immunity is also a potential mechanism of action of OVs, likely induced by tumor antigen uptake by antigen-presenting cells (APCs), such as dendritic cells (DCs), and the release of danger signals that stimulate innate immunity[1]. We hypothesized that OVs that express powerful activators of APCs will present exceptional ability to stimulate an anti-tumor immune response **to convert immune-desert to inflamed tumors** that are responsive to current immunotherapeutics. Using a type 5 adenovirus backbone, we generated a new OV that expresses high levels of a CD40 ligand (CD40L) and IFNβ with complementary mechanisms of action:

- Engagement of CD40 expressed on DCs by CD40L leads to acquisition of a crucial cross-priming function to activate CD8 T cells. MEM40 is a novel chimeric CD40L designed for stable cell surface expression. MEM40 has previously been evaluated in a non-replicating adenovirus serotype 5 vector (ISF35) and found to strongly synergize ICI in a mouse melanoma model[2].
- Type 1 IFNs, such as IFNβ, can function as direct activators of DCs and CD8 T cells. Type 1 IFNs also enhance expression of a host of immune function genes across multiple cell types, such as HLA/MHC and chemokines that attract DCs and T cells. Previous studies have also shown that CD40 ligation synergizes with type 1 IFNs to induce robust CD8 T cell responses.

Materials and Methods

We used a conditionally replicative type 5 adenovirus backbone that includes E1A, E1B and E3 viral genome deletions: (a) delta-24 (Δ24) E1A deletion results in tumor cell specific virus replication, (b) E1B 55kDa deletion allows enhanced replication in *TP53* mutant tumors, (c) E3 deletion mediates immune escape of the virus. This adenovirus backbone was used for the transgene-encoding viral constructs in our studies:

- GFP: CMV-GFP expression vector
- MEM-188: CMV-MEM40 (CD40L) expression vector
- MEM-288: CMV-MEM40 (CD40L) and SV40-human IFNβ dual transgene vector (Figure 1A)

CMV and SV40 promoter-driven expression cassettes were inserted either upstream of the E1A region or in the E3-deleted region, respectively. These viruses were tested against multiple human and mouse cancer cell lines *in vitro* and *in vivo*, including analysis in human xenograft and immunocompetent mouse tumor models following intratumoral injection.

References

(1) Bommareddy et al. 2018. Integrating oncolytic viruses in combination cancer immunotherapy. *Nat Rev Immunol* 18:498-513.

(2) Singh, M. et al. 2017. Intratumoral CD40 activation and checkpoint blockade induces T cell-mediated eradication of melanoma in the brain. *Nat Commun* 8:1447.

Results

Dual-armed oncolytic adenovirus MEM-288 generates strong oncolytic activity compared to control viruses
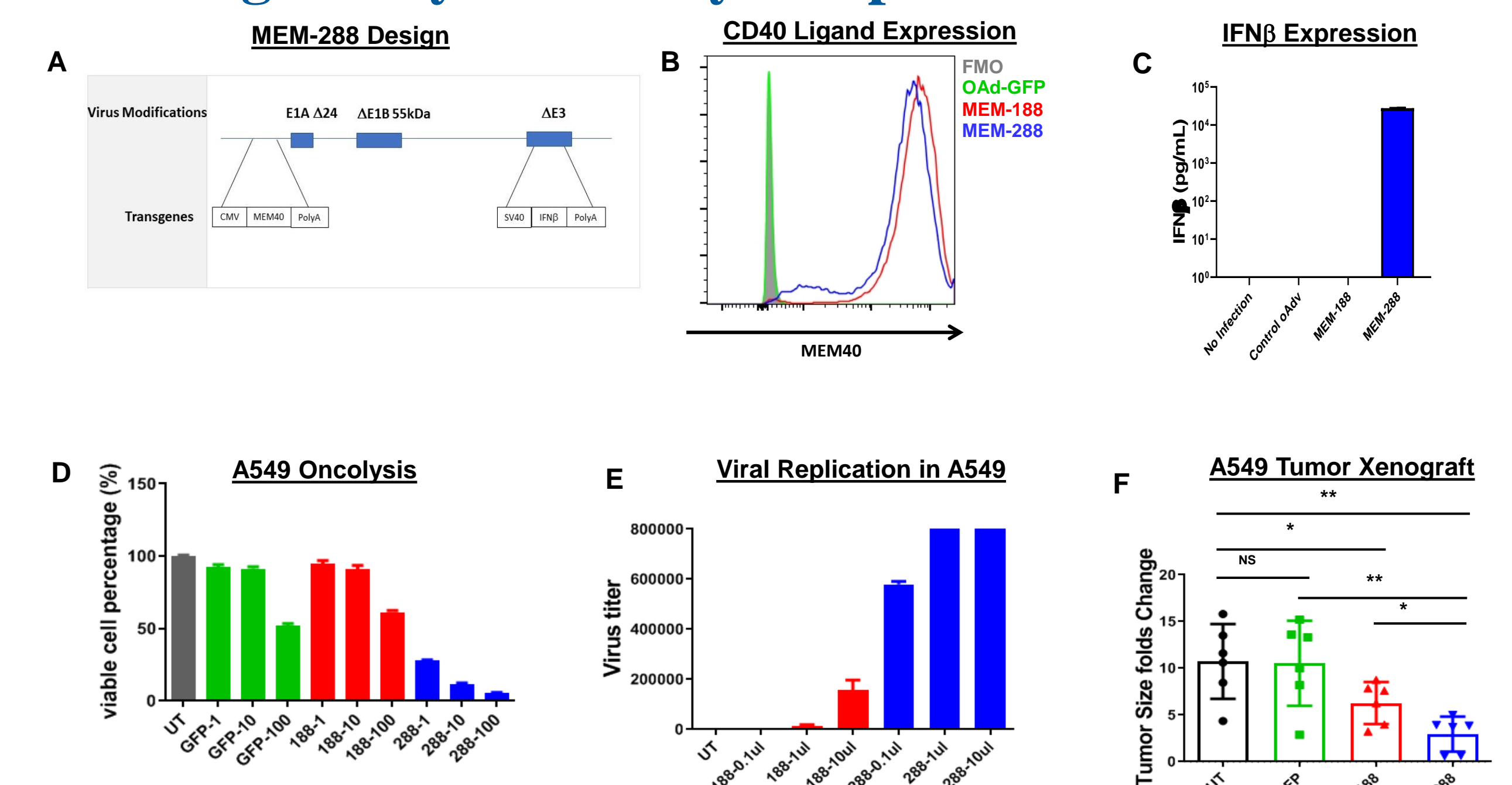

Figure 1. (A) The design of MEM-288. (B) A549 cells were infected with control GFP virus (oAd-GFP) or oncolytic MEM-188 or MEM-288 at MOI=250 for 2 days. MEM40 (CD40L) expression was determined by FACS. (C) IFNβ secretion was determined by ELISA following infection with indicated OV in A549 cells. (D) A549 cells were infected with control GFP virus, MEM-188 or MEM-288 at different MOIs for 2 days. Cell viability was determined by trypan blue staining assay. (E) 293AD cells were infected with harvested cell lysates of A549 infected at MOI=10 for 2 days and viral titers were determined by adenovirus titering kit. (F) 5e6 A549 cells were injected in flank of mouse. Mice then were injected with Ad-GFP, MEM-188 or MEM-288 at 10e9 pfu twice on D21 and D28. BLI was performed 14 days after the treatments. Statistical significance is indicated by p-values or as *p<0.05, **p<0.01, ***p<0.001. NS: not significant.

Selective oncolytic effect of MEM-288 in human cancer cells but not in human normal cells or mouse cancer cells
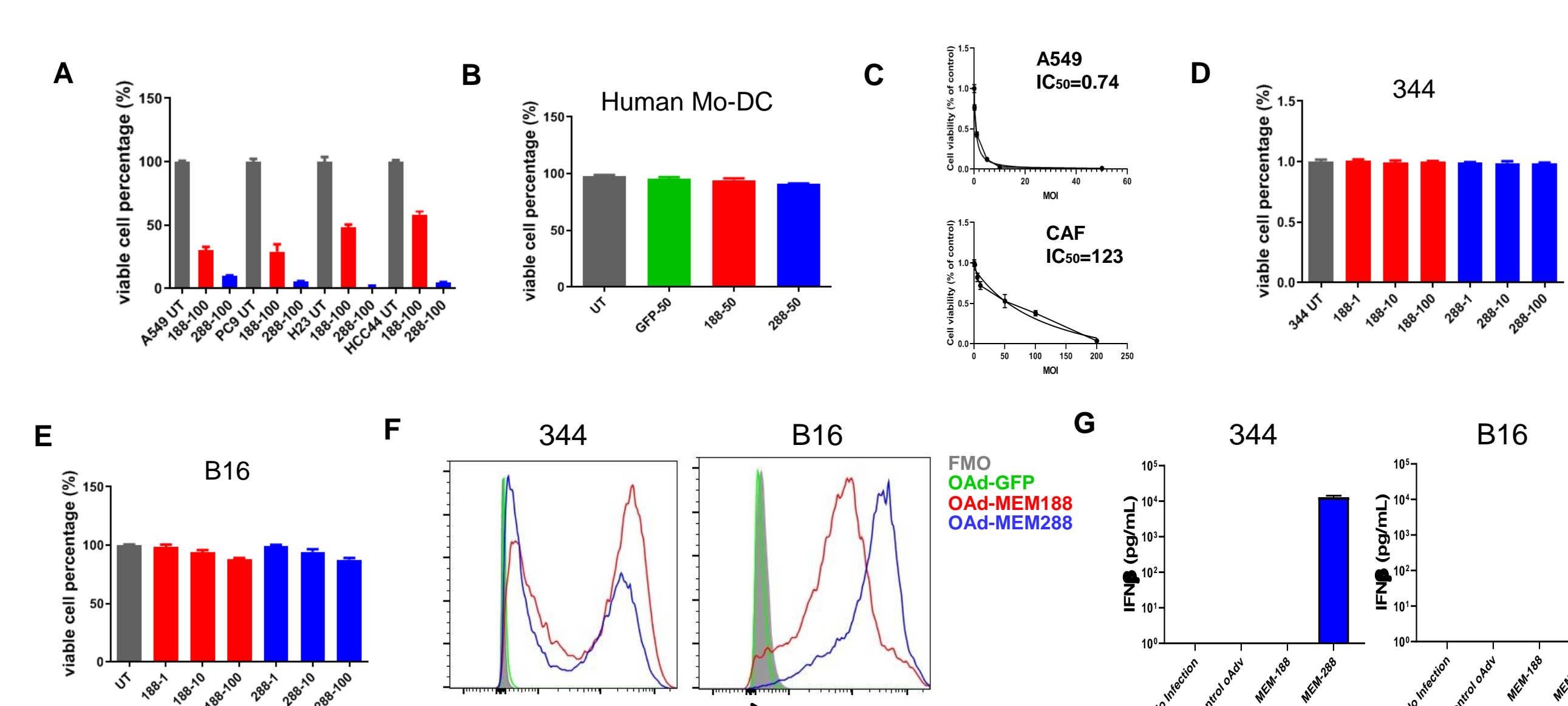

Figure 2. (A) Human lung cancer cell lines A549, PC9, H23 and HCC44 cells were infected with MEM-188 or MEM-288 at different MOIs for 2 days. Cell viability was determined by trypan blue staining assay. (B) Human CD14+ PBMC from healthy donor were purified by positive column selection and stimulated with 10ng/mL of GM-CSF and IL-4 for 6 days. On D4, cells were infected with oncolytic viruses at MOI of 50 and cell viability was determined by FACS on D6 on CD11c+ and MHC-II+ cells. (C) Same as (A) comparison of IC50 MOI in A549 and human cancer-associated fibroblasts. (D) Mouse 344 or (E) mouse B16 cells were infected with MEM-188 or MEM-288 as indicated at different MOIs for 2 days. Cell viability was determined by trypan blue staining assay. (F) 344 and B16 cells were infected with control GFP virus, MEM-188 or MEM-288 at MOI=250 for 2 days. MEM40 (CD40L) expression was determined by FACS and (G) IFNβ secretion was determined by ELISA following infection.

Oncolytic adenovirus MEM-288 inhibits tumor growth and markedly potentiates the abscopal effect in a melanoma model
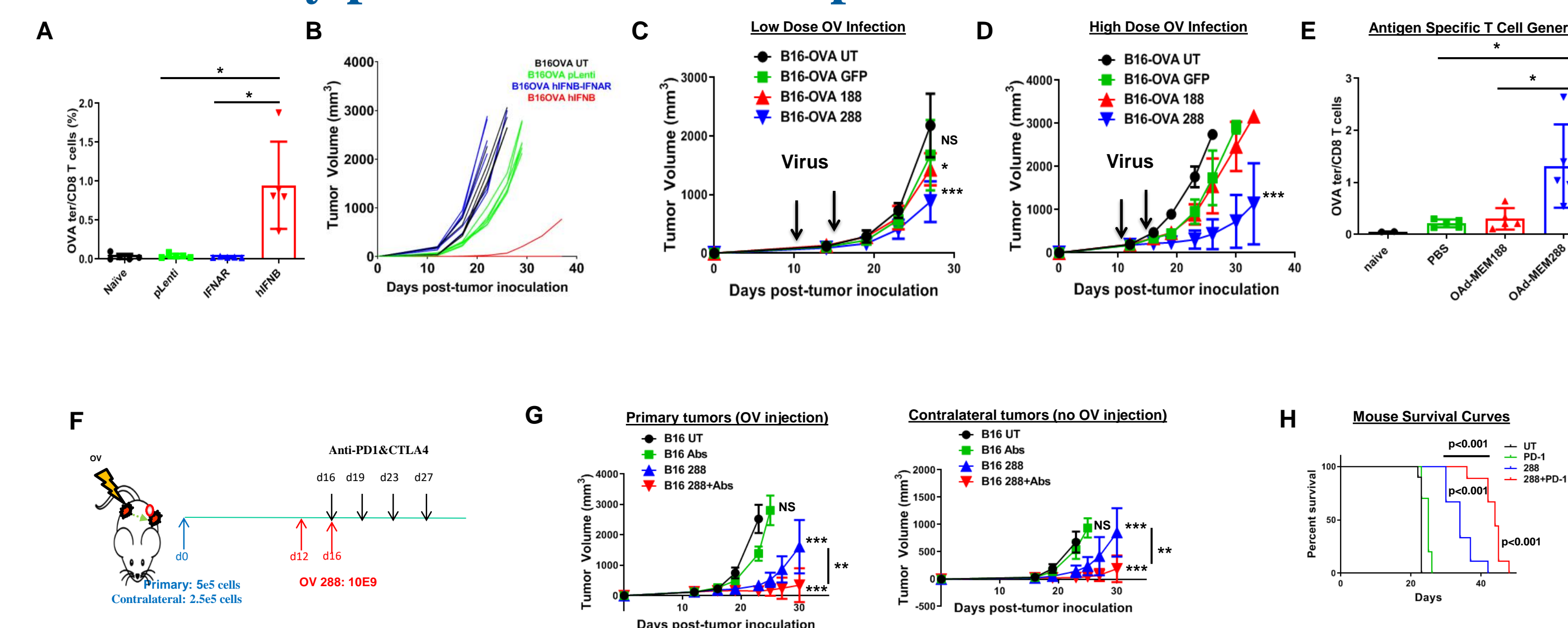

Figure 3. (A) Wild-type or IFNAR1-/- (IFNAR) C57BL/6 mice were subjected to two rounds of i.p. B16OVA-pLenti or B16OVA-IFNβ vaccine injection on D0 and 7. Percentage of OVA tetramer-positive cells in MHCII- and CD8+ cells in peripheral blood on D12 is shown. (B) Same mice as (A) except challenged with 3e5 live B16OVA cells on D21. Tumor growth was determined as indicated. These results indicate that human IFNβ has antitumor activity in mice. (C-D) C57BL/6 mice were inoculated s.c. with 5e5 B16OVA cells. On D12 and 16, these mice were subjected to two intratumoral injections of MEM-188 or MEM-288 at 10e8 (C) or 10e9 IU (D) showing dose response effects. Significance of tumor growth difference was calculated using two-way ANOVA. (E) Same as in (D) percentage of OVA tetramer-positive cells in MHCII- and CD8+ cells in peripheral blood on D20 is shown. (F) Treatment regimen in mice: C57BL/6 mice were inoculated s.c. with 5e5 B16-F10 cells on the primary site and with 2.5e5 B16-F10 cells on the contralateral site. These mice were injected with MEM-288 at 10e9 IU on D12 and 16 into primary tumors and with anti-PD-1 and CTLA-4 antibodies i.p. on D16, D19, D23 and 27. (G) Tumor growth was determined on the primary site and contralateral site as indicated. Significance of tumor growth difference was calculated using two-way ANOVA. (H) Kaplan-Meier Survival Analysis showing overall survival of the mice in the experiment in (F). Statistical significance is indicated by p-values or as *p<0.05, **p<0.01, ***p<0.001. NS: not significant.

Potent ability of oncolytic adenovirus MEM-288 to induce T cell activation and inhibit lung metastasis in a lung cancer model
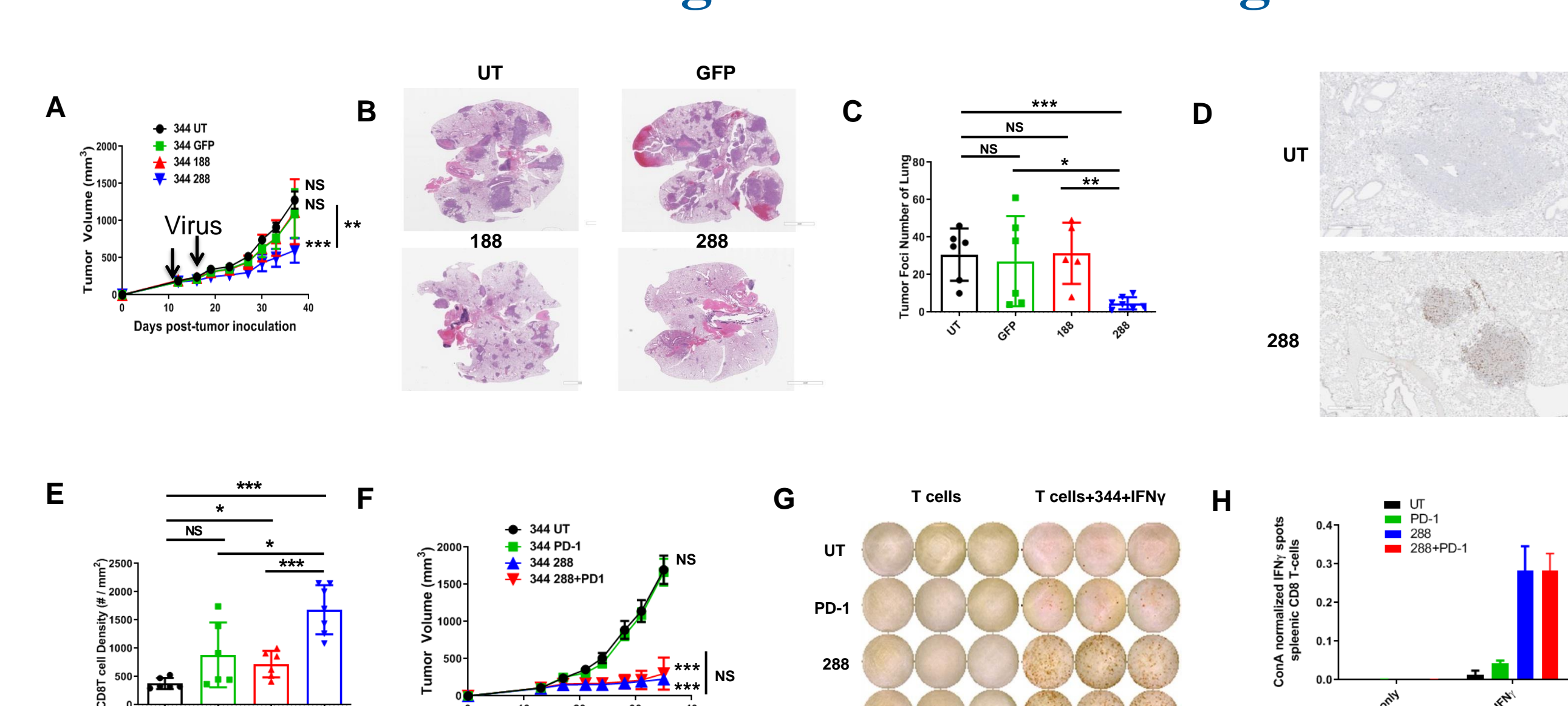

Figure 4. (A) 129 mice were inoculated s.c. with 5e5 344 cells on the flank and subjected to Ad-GFP, MEM-188 or MEM-288 at 10e9 IU on D12 and 16 into the tumors. Tumor growth was determined on the primary site as indicated. Significance of tumor growth difference was calculated using two-way ANOVA. (B) Typical H&E staining of tumors in lung metastasized from the flank tumor of the mice in (A) on D38. (C) Quantification of tumor burden of metastasis in (B) is shown. (D) Typical IHC staining of CD8α in the lungs of mice in (A). (E) Quantification of CD8α T cell density in tumor from (D) is shown. (F) Mice were inoculated s.c. with 5e5 344 cells on the flank and subjected to MEM-288 at 10e9 IU on D12 and 16 into the tumors and anti-PD-1 antibody i.p. on D16, D19, D23 and 27. Tumor growth was determined on the primary site as indicated. Significance is indicated compared to control groups using two-way ANOVA . (G) IFNγ ELISPOT from the spleen CD8 T cells of the mice in (F). (H) Quantification of ELISPOT results from (G) is shown as indicated. Statistical significance is indicated by p-values or as *p<0.05, **p<0.01, ***p<0.001. NS: not significant.

Conclusions

- MEM-288 exhibited high-level oncolytic activity in a broad range of human solid tumor cell lines, including several lung cancer lines.
- MEM-288 induced selective lysis of tumor cells compared to normal cell types, such as human dendritic cells and cancer-associated fibroblasts (CAFs).
- In immunocompetent mice, MEM-288 generated a highly efficacious immune response against both intratumoral injected and distant non-injected contralateral tumors in the B16-F10 melanoma model, including significantly greater inhibition of tumor growth with MEM-288 compared to the combination of anti-CTLA4 + PD-1. In addition, the combination of MEM-288 with anti-CTLA4 + PD-1 significantly enhanced anti-tumor activity.
- MEM-288 generated significantly better T cell tumor antigen-specific clonal expansion following intratumoral administration in the B16-OVA melanoma model compared to control OVs.
- Intratumoral administration of MEM-288 in the syngeneic mouse lung metastatic model (344SQ) demonstrated potent systemic anti-metastatic activity compared to control OVs. MEM-288 induced a strong T cell response resulting in tumors with high levels of tumor infiltrating lymphocytes (TILs).
- These positive preclinical data suggest MEM-288 is potent tumor-selective oncolytic virus with desirable mechanistic features that can be used as a single agent or in combination with ICI therapy in the clinical setting. A Phase I study of MEM-288 alone and in combination with anti-PD-1 in solid tumors is planned to start later this year.
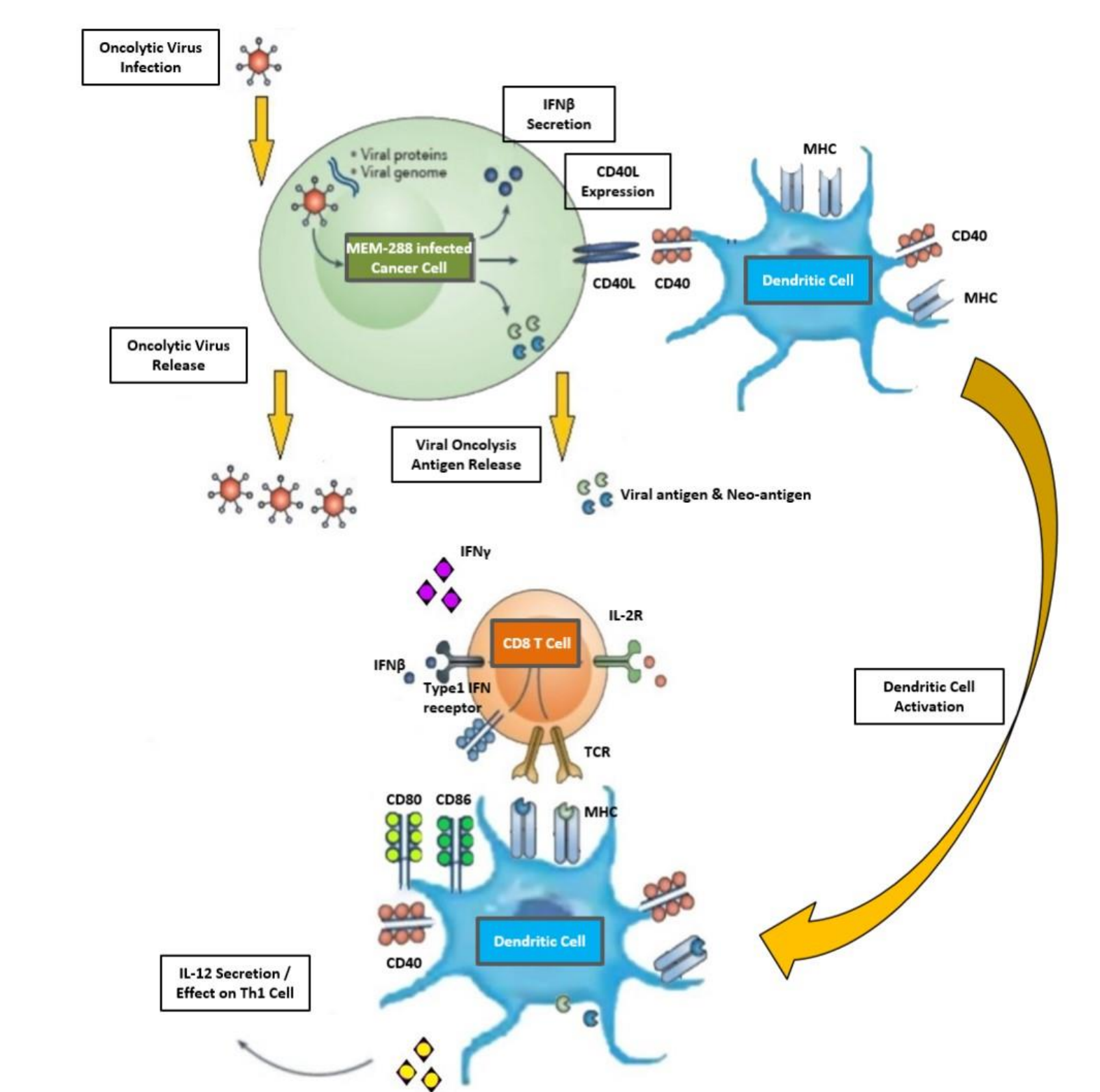
MEM-288 Mechanisms of Action

Figure 5. MEM-288 infection of cancer cells results in oncolysis to release free virus and tumor antigens. Dendritic cells uptake tumor antigens and receive CD40L and IFNβ activation signals from tumor cells. These activated DCs with high CD80/CD86/IL-12 expression serve as potent initiators of antitumor CD8 T cell activation. IFNβ produced by tumor cells may additionally enhance CD8 T cell clonal expansion.

Acknowledgements