

# Enhancing dendritic cell therapy with oncolytic adenovirus 3 coding for CD40L

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## Background

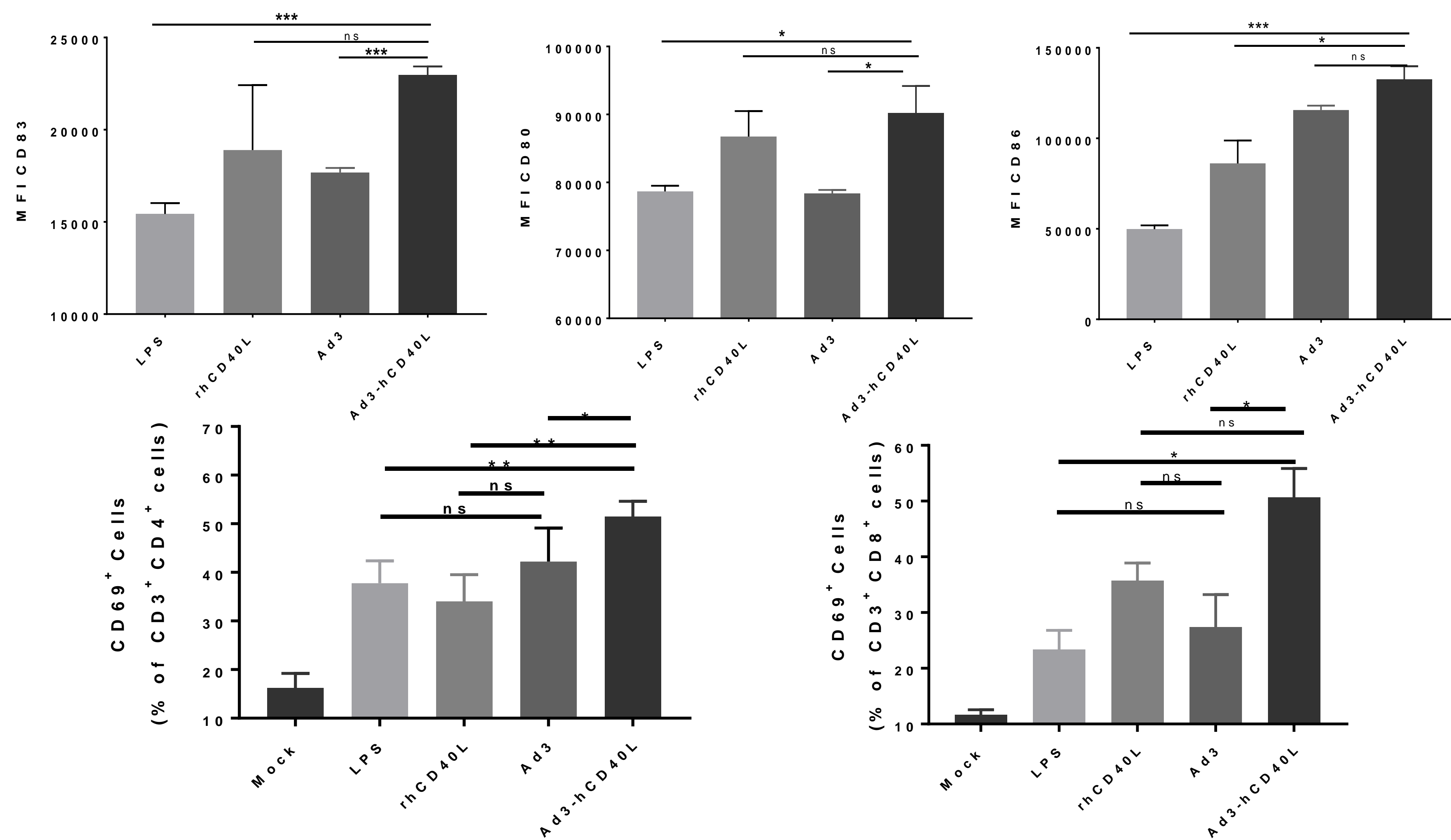
- ✓ Dendritic cell (DC) therapy is currently considered as a promising therapeutic option for treatment of cancers.
- ✓ However, tumor induced immunosuppression impairs the function of dendritic cells, eventually leading to tumor tolerance.
- ✓ Oncolytic adenoviruses cause biological effects which could be utilized to reverse the immunosuppression.

## Aims

- ✓ To assess the antitumor efficacy of CD40L coding virus *in vivo* in combination with DC therapy in the presences of human immune cells.
- ✓ To study the correlation between the adenovirus expressing CD40L and DCs combination treatment with induction of antitumor immune response.

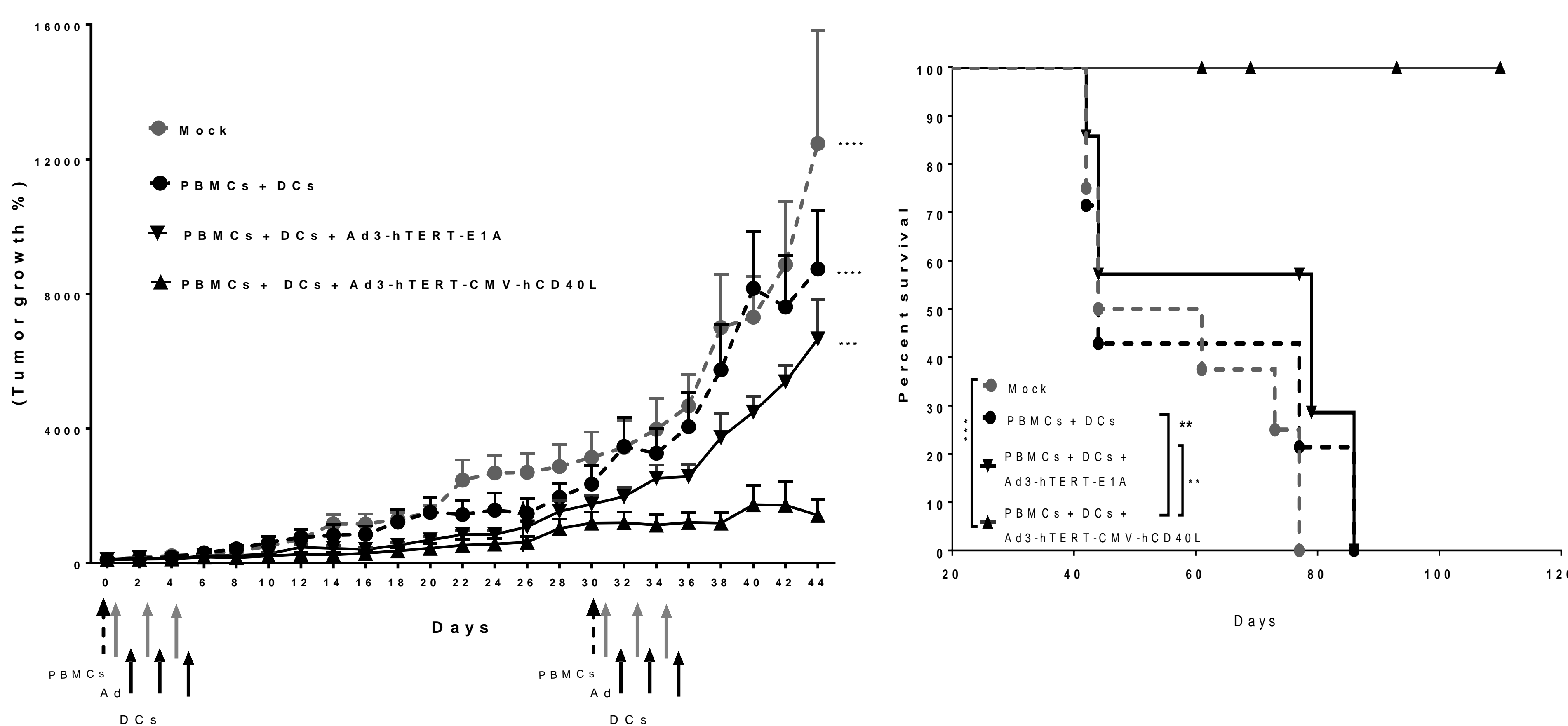
## Results

### 1. Ad3-hTERT-CMV-hCD40L infected tumor cells induces DCs maturation, resulting T cell stimulation in a mixed lymphocyte reaction *ex vivo*



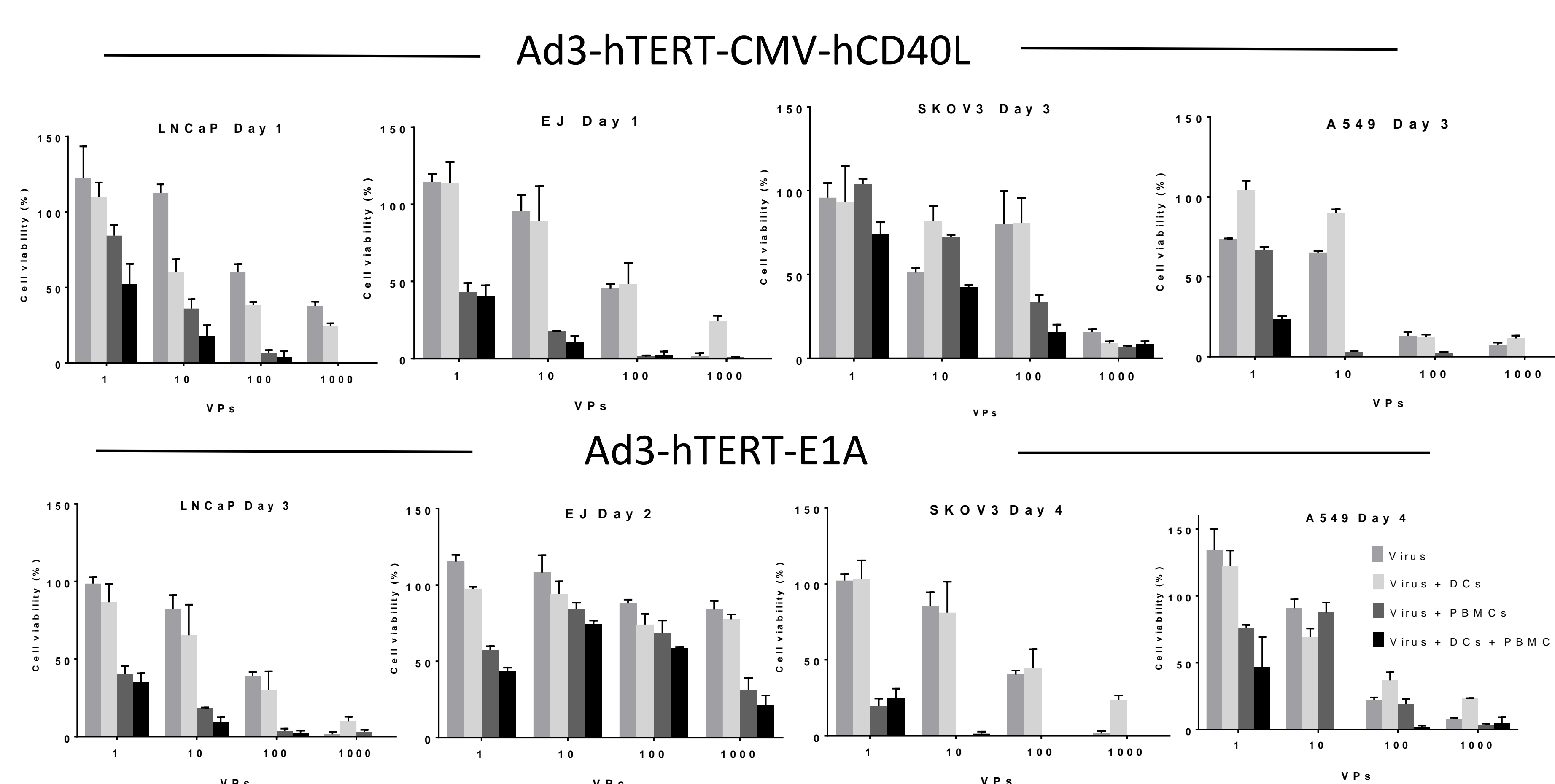
**Figure 1:** A549 cells were infected with viruses or left uninfected. After 18 h., cells were washed with PBS before adding immature dendritic cells. After 48h., co-culture DCs were assayed for maturation or co-cultured with T cells. T cell activation was assessed 24hr later by flow cytometry. LPS, lipopolysaccharide, rhCD40L: recombinant hCD40L protein, Ad3-hCD40L and Ad3: cells infection with Ad3-hTERT-CMV-hCD40L and Ad3-hTERT-E1A viruses, respectively.

### 3. Antitumor efficacy of Ad3-hTERT-CMV-hCD40L and dendritic cell combination in a mice humanized with human peripheral blood mononuclear cells (PBMCs).



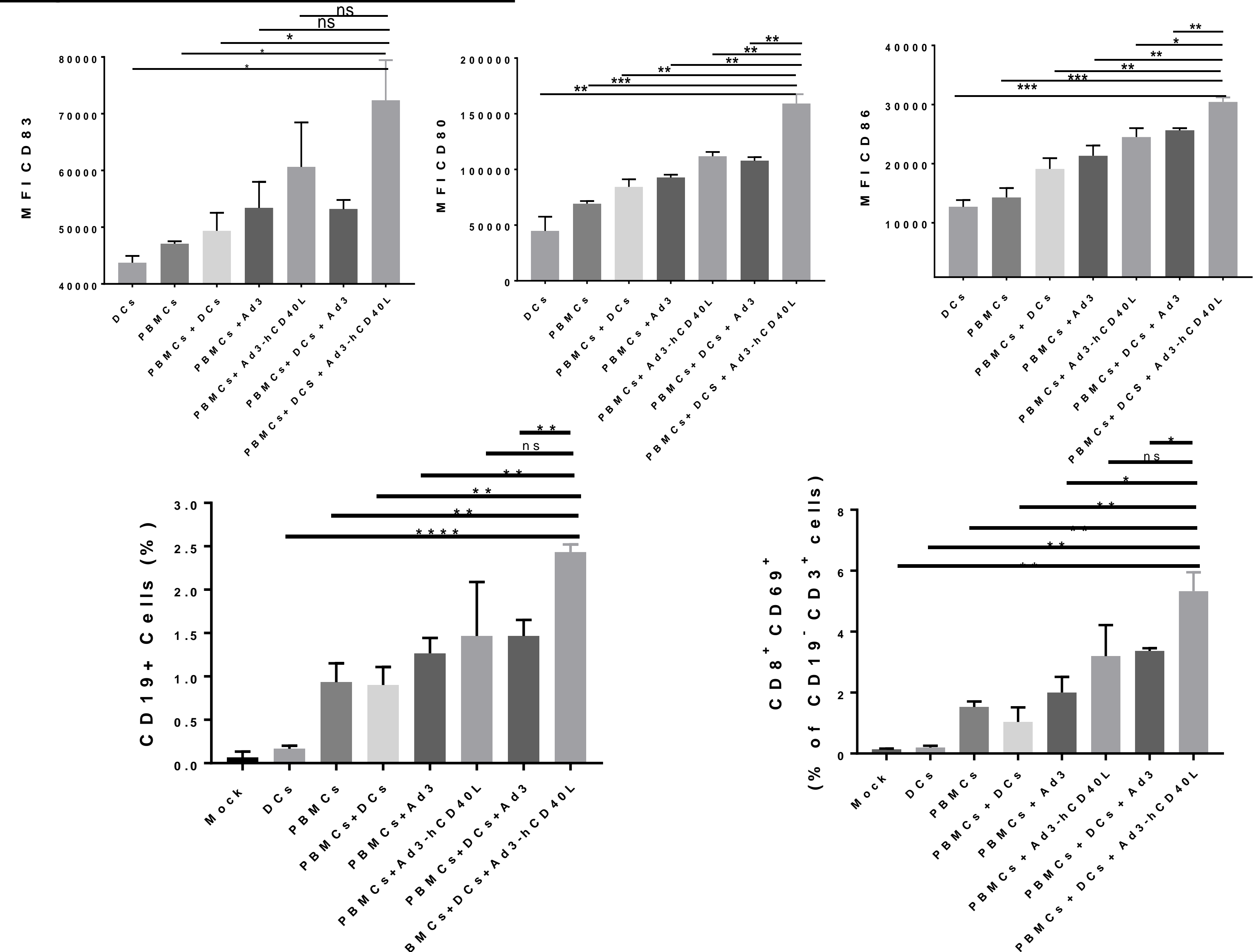
**Figure 3:** A549 tumors were injected subcutaneously in immunodeficient mice. Viruses and dendritic cells were injected intratumorally three times. PBMCs were injected intravenously once. Tumor growth is expressed as percentage increase from first day of virus injection. Overall survival and statistic significances.

### 2. Combination of Ad3-hTERT-CMV-hCD40L, DCs and human peripheral blood mononuclear cells PBMCs kill tumor cells *ex vivo*



**Figure 2:** Tumor killing potency of Ad3-hTERT-CMVhCD40L or Ad3-hTERT-E1A, DCs and PBMCs was assessed in LNCaP, EJ, SKOV3 and A549 cells. Cell killing was assessed at different time points (after adding DCs and PBMCs in co-culture) with MTS assay.

### 4. Combination of Adenovirus coding CD40L with DCs in mice humanized with PBMCs enhances anti-tumor immune response *in vivo* in tumors



**Figure 4:** Immune response in tumor microenvironment. Median fluorescence intensity (MFI) of the channel for CD83, CD80 and CD86 of CD11c + population. Percentage of CD19 + B cell population and CD8 + CD69+ lymphocytes of parent population of CD19-CD3+ cells.

## Conclusions

- ✓ Ad3-hTERT-CMV-hCD40L infected tumor cells induces maturation of human DCs which in turn required for T cell activation *ex vivo* and *in vivo*.
- ✓ We have shown promising anti-tumor immune responses suggesting that adenovirus coding for CD40L successfully enables dendritic cell therapy.
- ✓ Ad3-hTERT-CMV-hCD40L is promising for translation into human trials. In particular, this virus could enable successful dendritic cell therapy in cancer patients.