Genetic Modification for Immune Evasion

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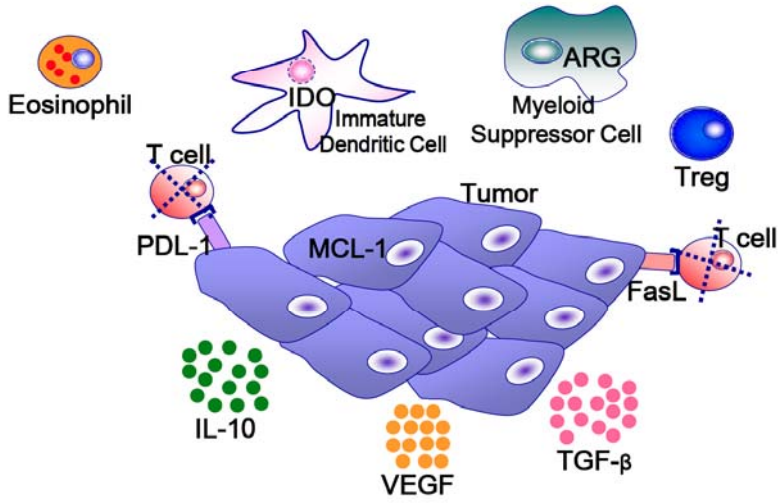
T-cell Therapy

'T-cell Therapy has shown promise in clinical studies'

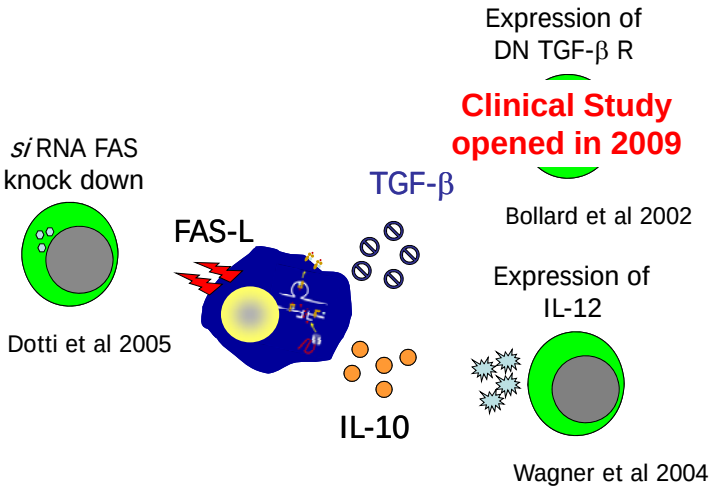
Suboptimal T-cell products: sensitive to the immunosuppressive Tumor environment



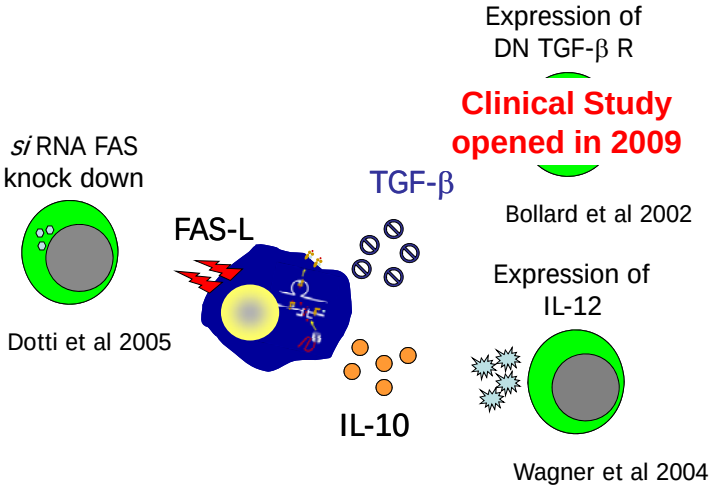
Genetic Modification of T cells

- Render T cells resistant
  - to immune evasion mechanisms employed by tumors
- Improve T-cell function
  - cytokines/cytokine receptors
  - anti-apoptosis genes
  - silencing negative regulators .....
- Antigen-specific T cells
  - Chimeric antigen receptors (CAR)
  - αβ TCR

Tumor cells secrete factors to inhibit immune cells

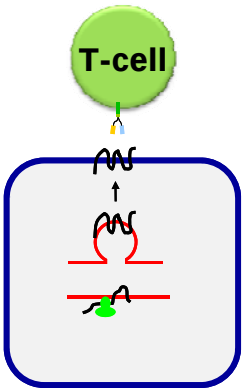


Tumor cells have MHC processing defects & antigen expression is heterogeneous

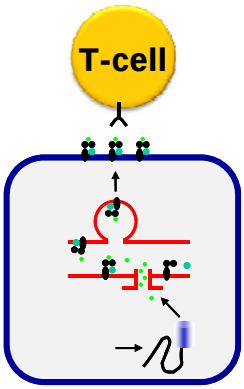


CAR vs αβ TCR T cells

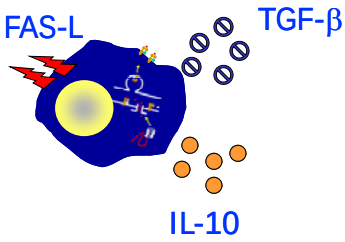
CAR T cells



αβ TCR T cells



CAR vs αβ TCR T cells



CAR T cells to overcome immune evasion

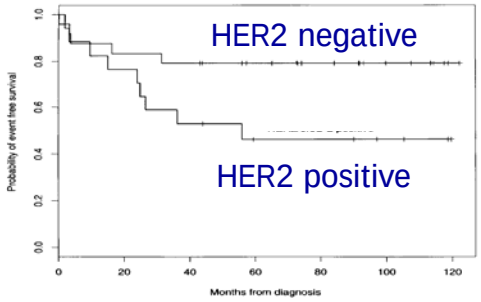
- Targeting cell surface antigens
- Targeting multiple antigens
- Targeting inhibitory stromal cells

Rationale for immunotherapy for osteosarcoma

- Prognosis remains poor for patients with
  - Metastatic disease (< 20% survival)
  - Central tumors (< 35% survival)
  - Relapsed disease
- Significant acute toxicities
- Long-term effects
  - Disability after surgery

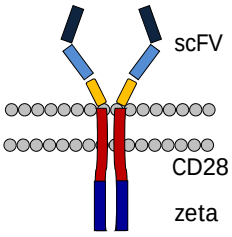
HER2 expression and prognosis

- Member of the EGFR family of receptors
- Expressed by up to 80% of primary osteosarcoma
- Important for malignant phenotype
- Worse overall survival rates



(Gorlick et al. JCO 1999)

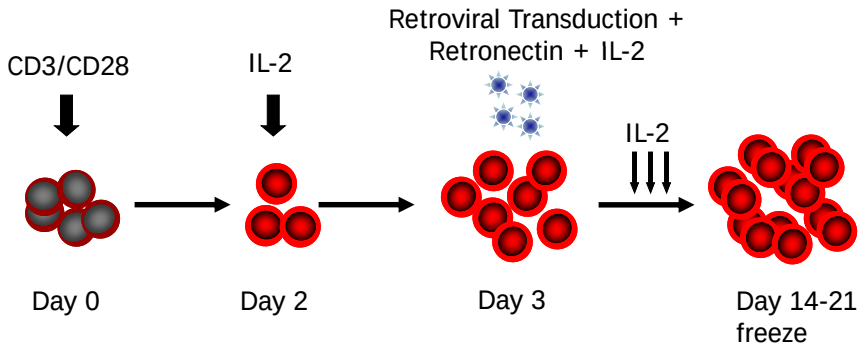
2nd Generation HER2-specific CAR



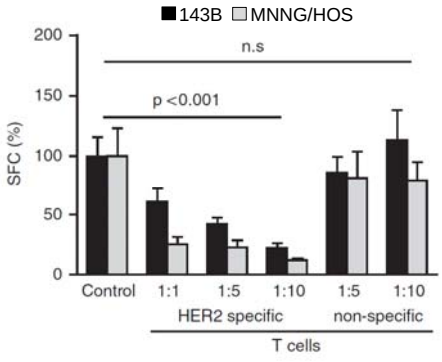
GALV or RD114 pseudotyped retroviral particles

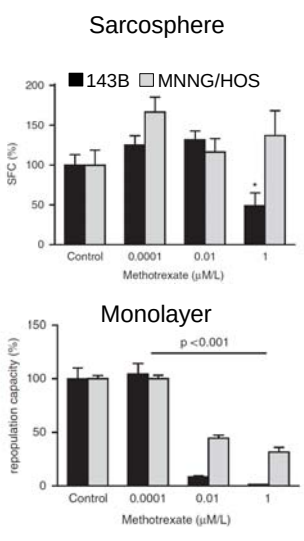
(Pule et al, Mol Thr 2005; Ahmed et al, Mol Thr 2009)

Generation of CART cells by retroviral transduction



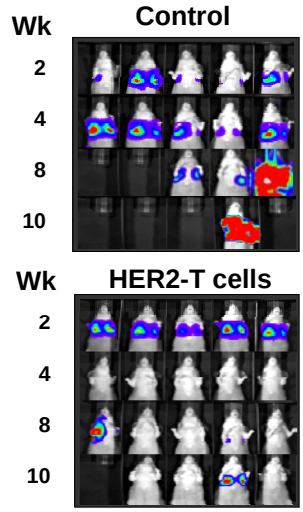
HER2-CAR T cells inhibit 2ndary Sarcosphere formation

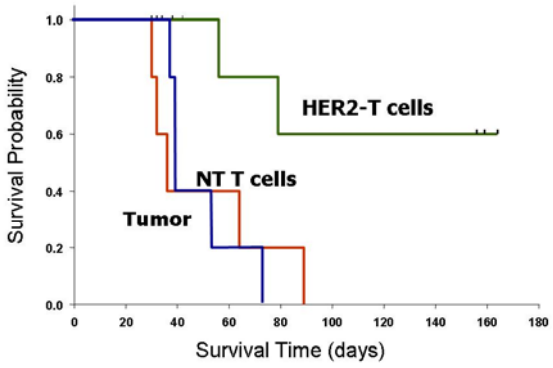




(Rainusso et al; Cancer Gene Therapy 2012)

Antitumor activity in metastatic Lung Model





(Ahmed et al. Mol Ther 2009)

Safety concerns with HER2-CAR T cells

- 6 patients have received 'conventional' HER2-specific T cells 1 - $4x10^{10}$ cells with no side effects
- 2 patients have received HER2-CAR T cells
  - 1^{st} : $2x10^{7}$ cells: no immediate toxicity
  - 2^{nd}: $1x10^{10}$ cells post nonmyeloablative conditioning: developed ARDS and died

(Bernhard et al Cancer Immunol Imm 2008, Disis et al JCO 2009, Morgan et al Mol Thr 2010)

T-cell Therapy for HER2-positive Osteosarcoma

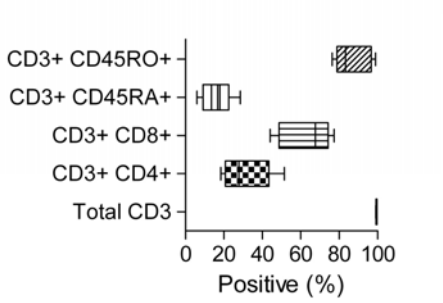
- To determine the safety and antitumor activity of HER2-CAR T cells
- 9 Dose levels: **$1x10^4$** to $1x10^8$ cells/m^2

Characteristics of infused patients

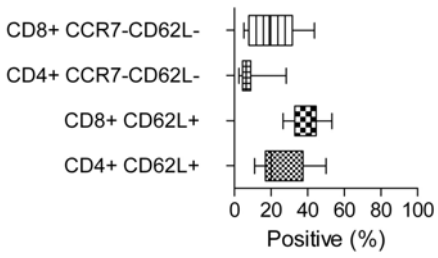
- 9 females, 6 males
- Median age 17.3 (7 – 29)
- 13 OS, 1 EWS, 1 DSCRT
- Disease status
  - 11 lung mets
  - 1 lung and bone mets
  - 1 lung and extraosseous mets
  - 1 Liver mets
- All patients had failed multiple lines (>4) of salvage therapy

Phenotype of GMP grade HER2-CAR T-cells

Frequency of CD4 & CD8 T-cell subsets

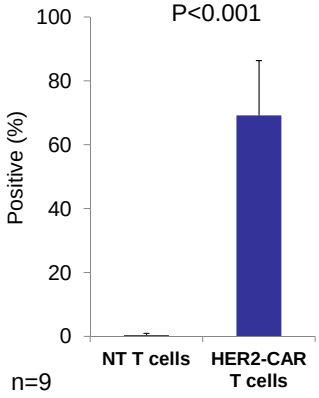


Frequency of CD3+CD45RO+ Effector & Central memory T cells

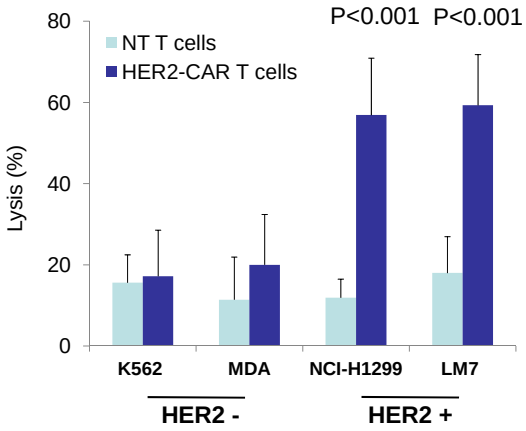


Characterization of GMP grade HER2-CAR T-cells from OS patients

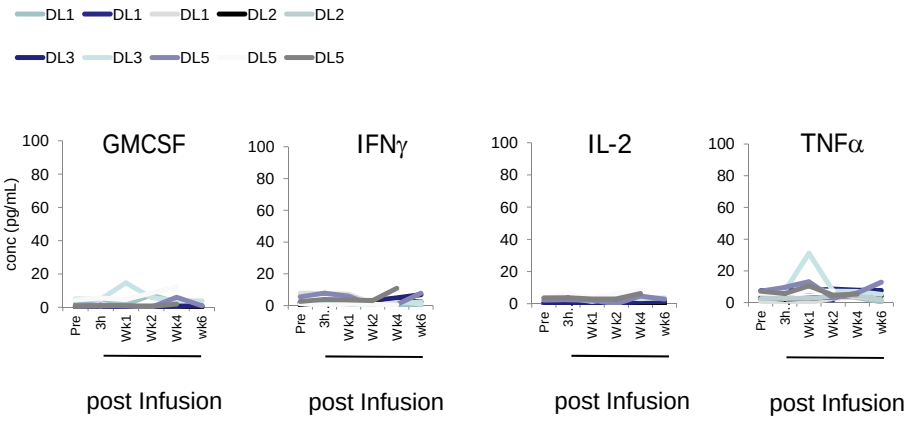
Transduction Efficiency



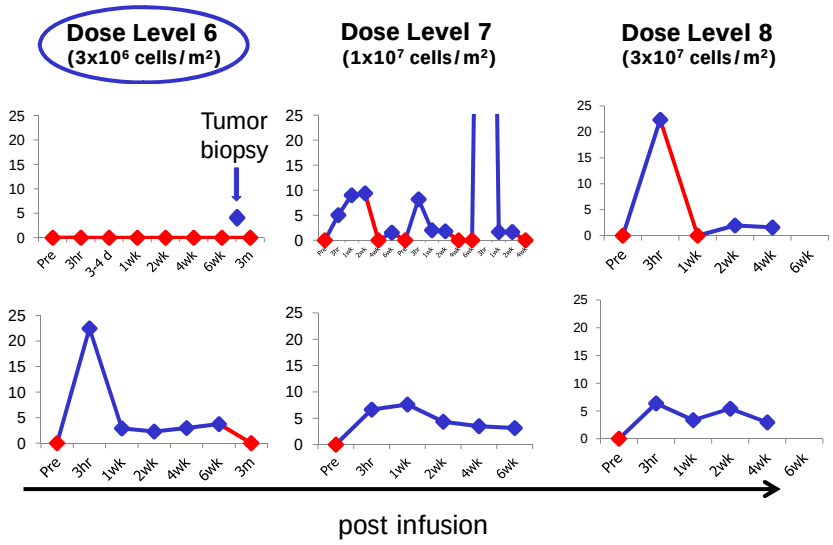
Cytotoxicity



No increase in pro-inflammatory cytokines post infusion

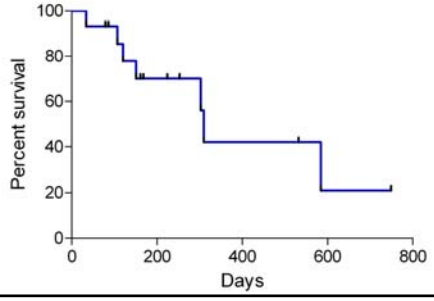


In vivo persistence of adoptively transferred HER2-CAR T cells



Clinical Outcome

- 4 patients with stable disease
  - 1 patient for 6 weeks (then had tumor resected)
  - 1 patient for 12 weeks (then had tumor resected)
  - 1 patient for 4 months
  - 1 patient for 5½ months (ongoing)
- 11 patients with progressive disease



Conclusions: T-cell Therapy for sarcoma

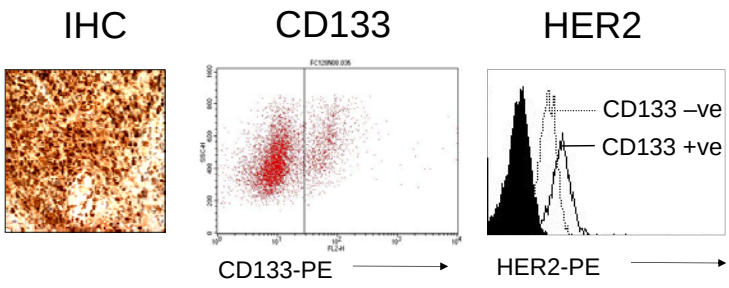
- HER2-CAR T cells
  - Have antitumor activity in preclinical models
- Adoptive transfer of HER2-CAR T cells in humans
  - Evaluated cells doses safe (up to $3x10^7/m^2$)
  - Limited T-cell persistence
- Plan
  - Clinical: Escalate HER2-CAR T-cell dose to $1x10^8/m^2$
  - Preclinical: Improve CAR; better preclinical models

CAR T cells to overcome immune evasion

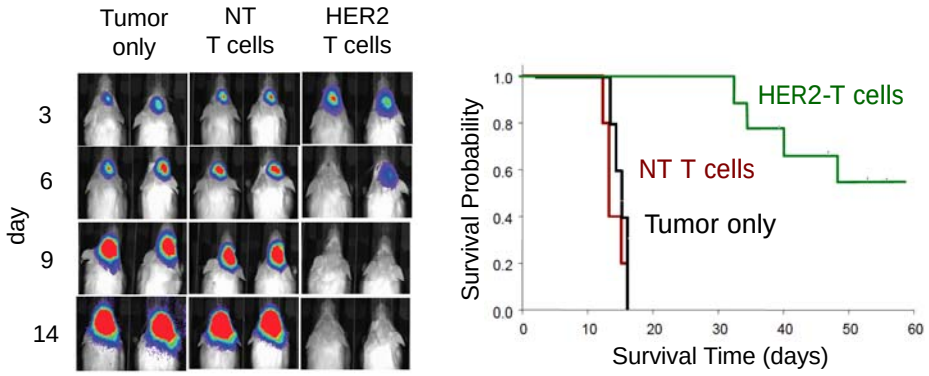
- Targeting cell surface antigens
- Targeting multiple antigens
- Targeting inhibitory stromal cells

HER2 as an Immunotherapy target for GBM

- ~ 70% of GBM positive HER2
- HER2
  - promotes malignant phenotype
  - expressed in CD133+ glioma initiating cell population



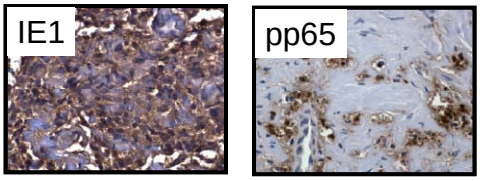
HER2-specific T cells induce regression of autologous GBM



(Ahmed et al, Clin Can Res 2010)

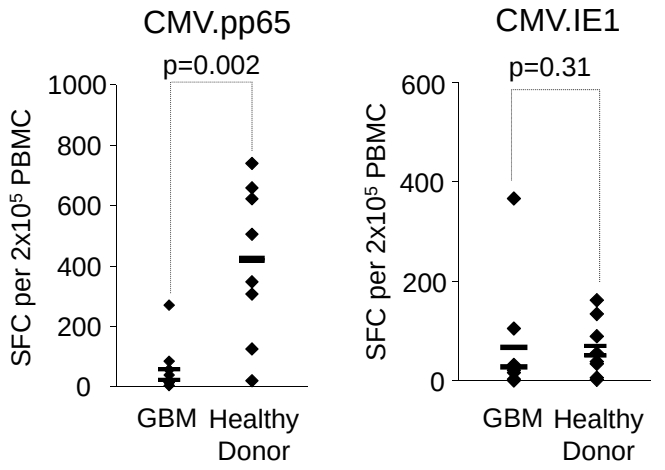
CMV as an Immunotherapy target for GBM

- Majority of GBMs are positive for CMV IE1 and pp65



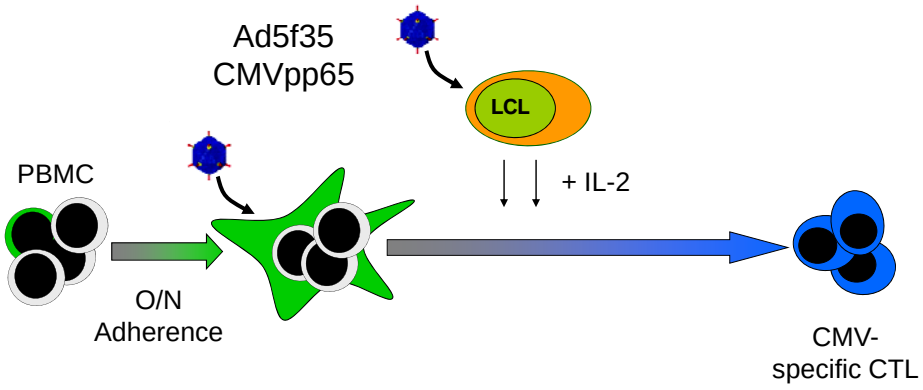
- Phase II adjuvant pp65/DC vaccine study:
  - Safe
  - Induction of pp65-specific T-cell responses
  - Prolonged survival in comparison to historic controls

GBM patients have decreased T-cell responses to pp65



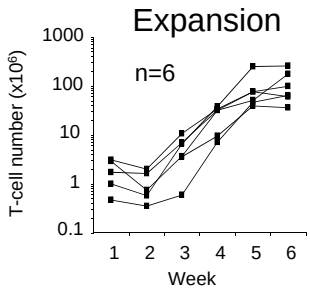
(Ghazi et al, JIT 2012)

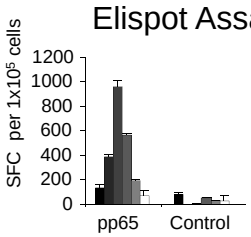
Generation of CMV-specific CTL using Ad5f35 Vectors

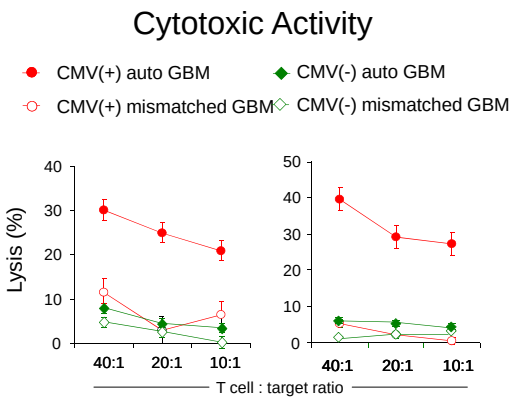


(Leen et al, Nat Med 2006)

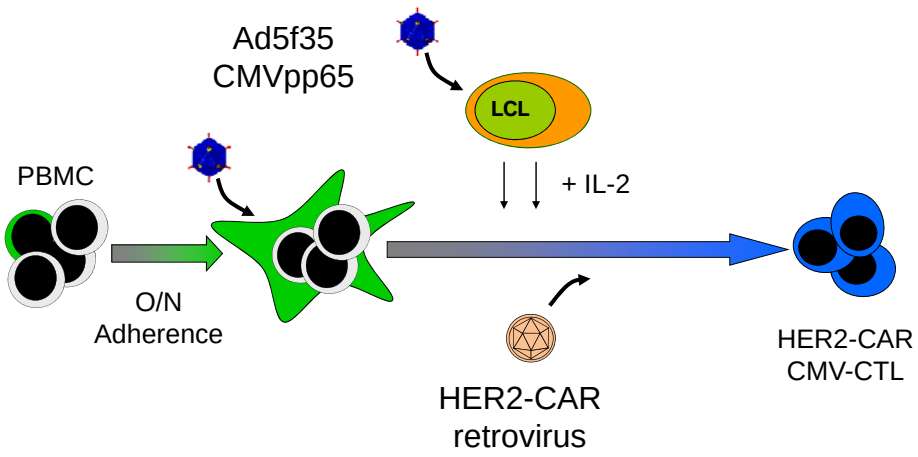
Generation of CMV-specific T cells from GBM patients



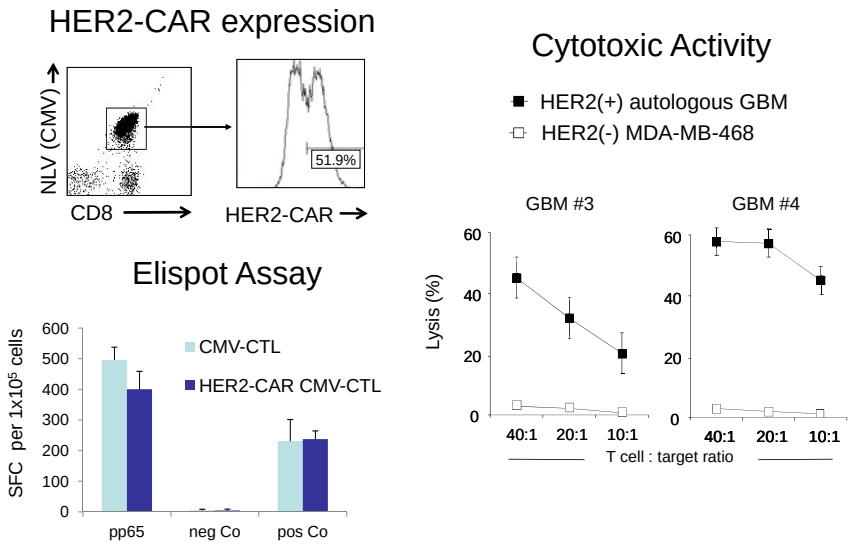




Generation of HER2-CAR CMV-specific CTL



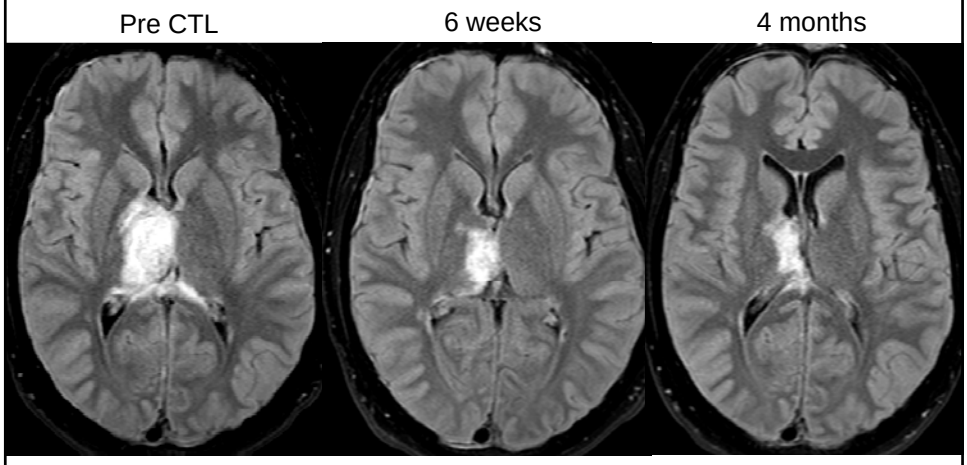
Characterization of HER2-CAR CMV-specific T cells



Characteristics & Outcome of infused patients

- 4 patients with recurrent GBM
- Cell dose: $1x10^6/m^2$
- Outcome:
  - 2 progressive disease
  - 1 stable disease for 4½ months
  - 1 partial response

Clinical response post T-cell infusion



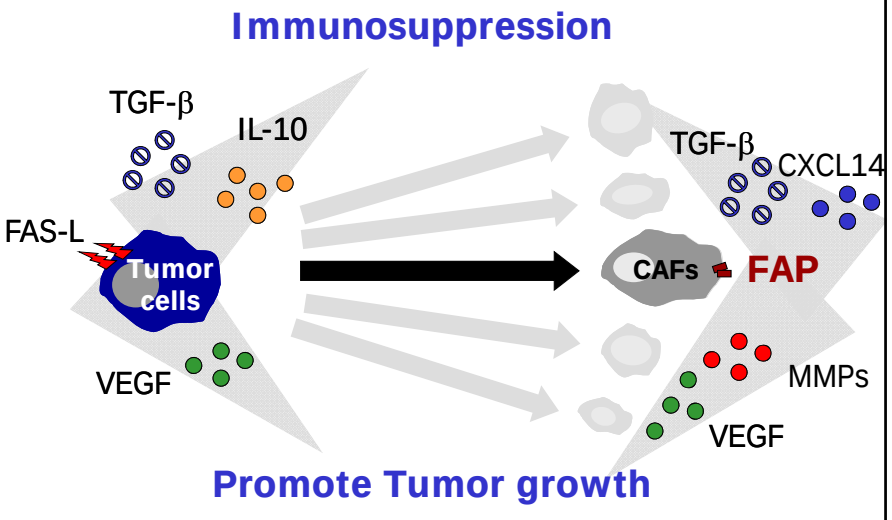
Conclusions: T-cell Therapy for GBM

- HER2 and CMV antigens are expressed in GBM
- Generation of clinical grade HER2/CMV-specific T cells is feasible
- Clinical Trial:
  - Safe data so far encouraging
- Plan:
  - Escalate HER2/CMV-specific T-cell dose to $1x10^8/m^2$

CAR T cells to overcome immune evasion

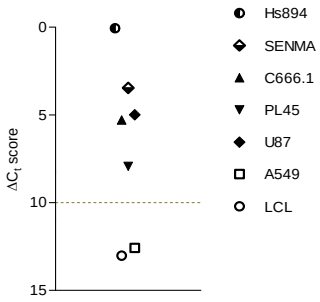
- Targeting cell surface antigens
- Targeting multiple antigens
- Targeting inhibitory stromal cells

CAFs play critical role in tumorgenesis

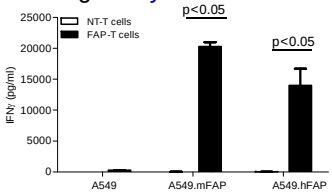


Characterization of FAP-CAR T cells

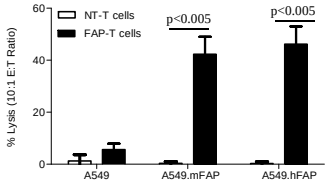
FAP-expression in cancer cell lines



FAP-CAR T cells recognize FAP+ targets: cytokine secretion

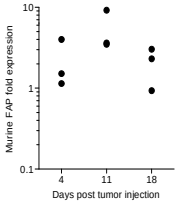


FAP-CAR T cells recognize FAP+ targets: cytolytic activity

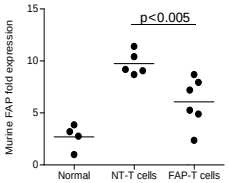


FAP-CAR T cells have antitumor activity *in vivo*

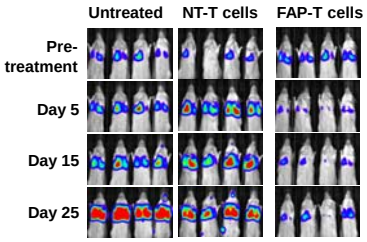
In vivo induction of murine FAP

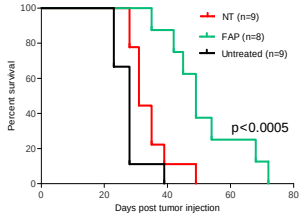


FAP-CAR T cells target FAP+ cells in vivo

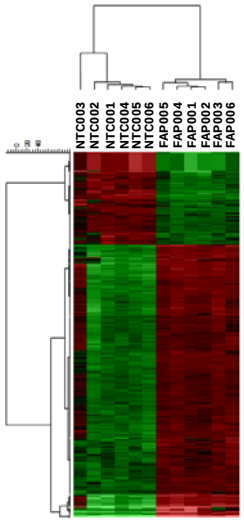


FAP-CAR T cells have antitumor activity *in vivo*





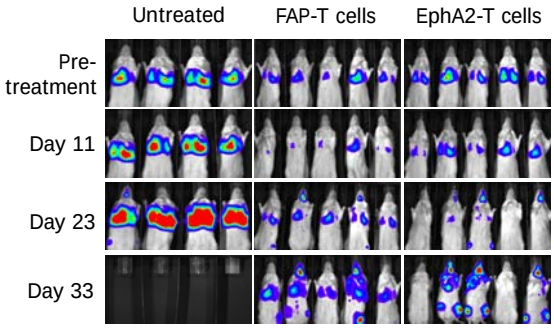
Targeting CAFs induces expression of pro inflammatory genes



Pathway analysis - enrichment of genes involved in

- chemokine & cytokine-cytokine receptor interaction
- toll-like receptor pathway
- natural killer cell mediated cytotoxicity
- Jak-STAT pathway
- B-cell receptor signaling
- antigen processing and presentation pathway
- complement and coagulation cascades

Combining FAP-CAR T cells with tumor-specific T cells enhances antitumor effects



Conclusions: Targeting tumor stroma

- CAFs contribute to immunosuppressive tumor environment
- Targeting CAFs results in antitumor effects and induces expression of pro-inflammatory genes
- Targeting CAFs and tumor cells results in enhanced antitumor effects

Conclusions

Tumors pursue a gamut of immune evasion mechanisms

BUT

Genetic modifications holds the promise to engineer T cells that are resistant to these ploys

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