Comparison of CAR-T Cell Therapy Generations for the Treatment of Glioblastoma

Thousand Oaks High School
AP Research- STEM

Introduction-

Glioblastoma and CAR-T cells

Glioblastoma- solid tumor in brain or spinal cord

- **80%** of malignant brain tumors
- **3** of every **100,000**
- Symptoms
  - Seizures
  - Vomiting
  - Bowel/bladder problems
  - Neurologic deficits

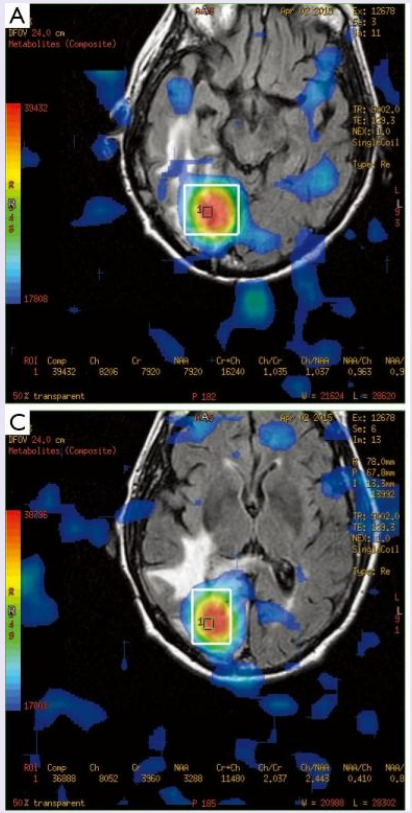


FIgure 1. Imaging of an area of interest, denoting a possible glioblastoma tumor in colored area

Treatments for glioblastoma

- Surgery
- Radiation therapy
- Chemotherapy
  - temozolomide (Temodar)

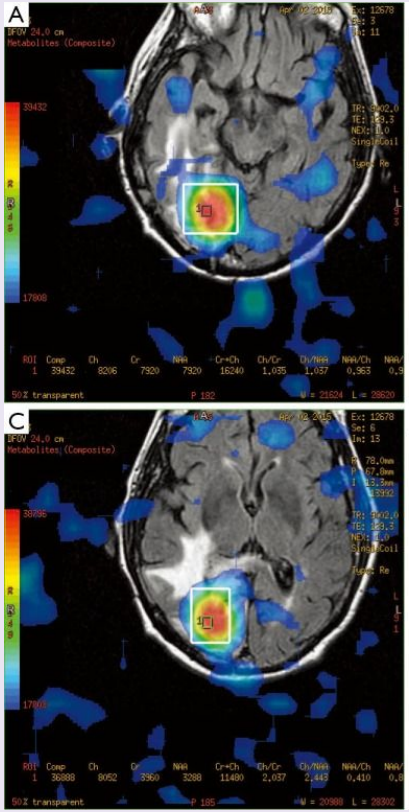
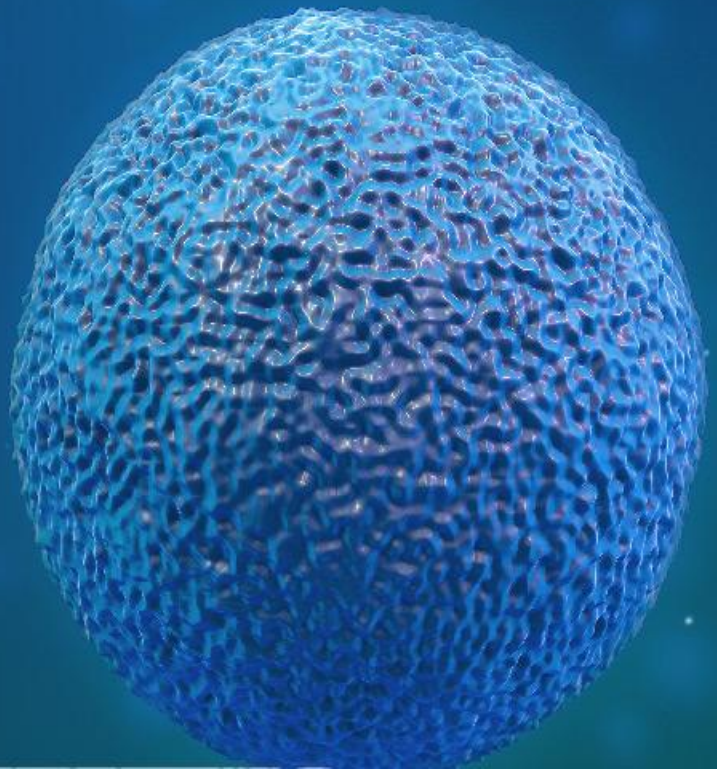


FIgure 1. Imaging of an area of interest, denoting a possible glioblastoma tumor in colored area

Immunotherapy

- Uses immune system to target & treat cancers
  - Vaccines
  - Tumor infiltrating lymphocytes (TIL)
  - TCR receptors
  - CAR-T cell therapy



Chimeric Antigen Receptor (CAR)-T cells

- Immune cells engineered with proteins to target tumors
- Already approved to treat liquid tumors:
  - Leukemia- Kymriah (*tisagenlecleucel*)
  - Lymphoma- Kymriah & Yescarta (*axicabtagene ciloleucel*)

CAR-T cell therapy (cont.)

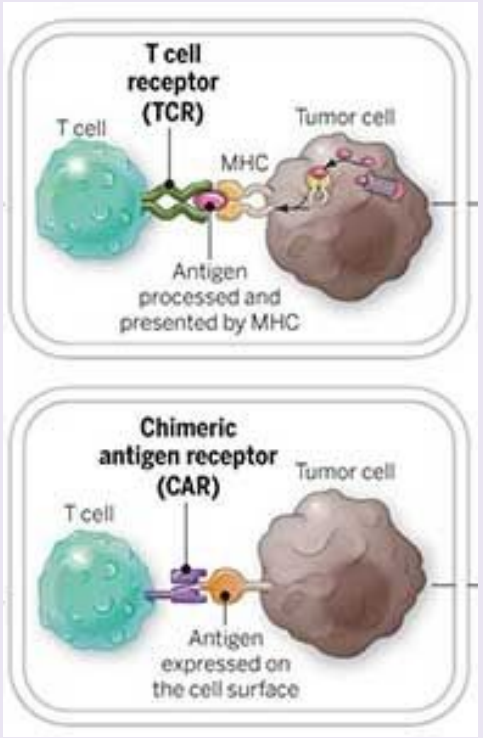


FIgure 2. Comparison of a T-cell and a CAR-T cell in detecting tumor cells. Both require detection of antigens

- Target receptors- **Antigens**
  - Mutation-associated neoantigens (MANA)
    - Epidermal Growth Factor Receptor variant 3 (EGFRvIII)
  - Tumor associated antigens (TAA)
- Varying antigen frequency
- Ideal target:
  - Eliminate most tumor cells
  - Avoid healthy cells

CAR-T cell Generations

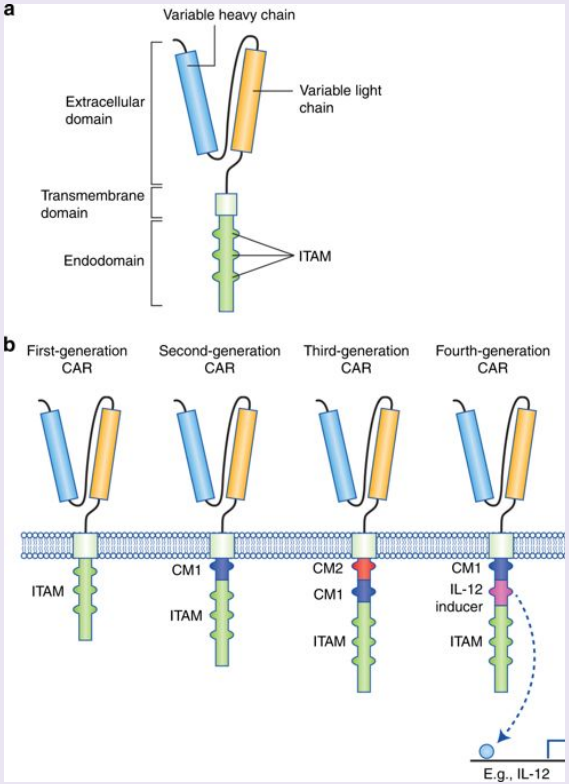


FIgure 3. Comparison of CAR structures for each generation, beginning with core structure

- Generations
  - **1st-** single CD3ζ intracellular domain
  - **2nd-** added costimulatory domain
  - **3rd-** added 2nd costimulatory domain
  - **4th (TRUCKs)-** added protein (IL-12)

Problems with CAR-T cells

- Difficult to treat solid tumors
  - Hostile tumor microenvironment
  - Heterogeneous gene expression

Purpose

To investigate a more effective treatment for glioblastoma using CAR-T cell therapy

Research Question

What generation of CAR-T cell therapy would be most effective to target glioblastoma?

Hypotheses

Alternative hypothesis-
New generation CAR-T cells perform significantly better than past generations in reducing glioblastoma tumor volume

Null hypothesis-
There is no difference between the different CAR-T cell generations in reducing glioblastoma tumor volume

Methods-

Systematic Literature Review

Methods-

Criteria

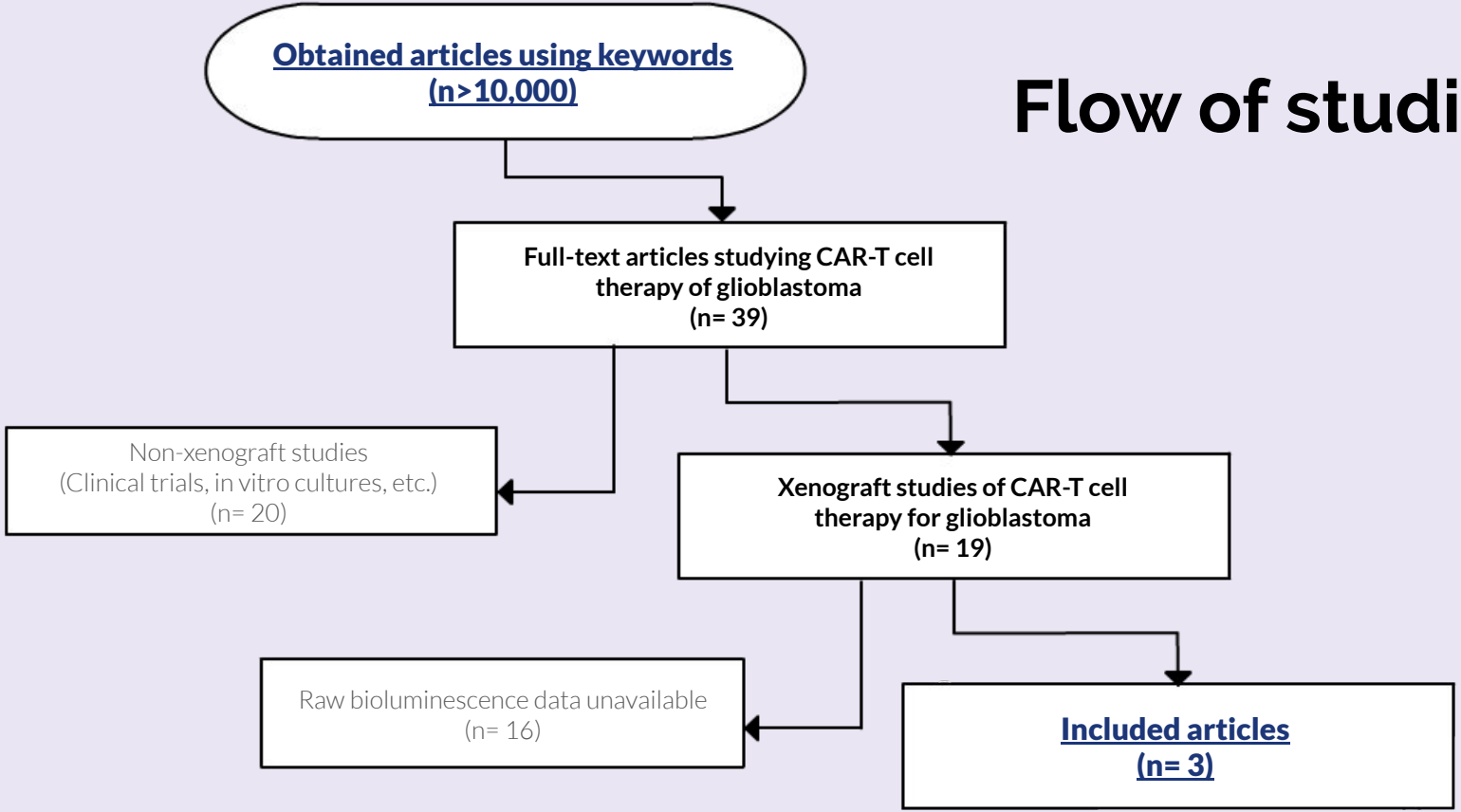
Sources	Criteria	Key Search Terms
• PubMed • EBSCOhost • Google Scholar	• Clinical trials • Xenograft models • Glioblastoma cell lines • CAR-T cell therapy	• “CAR-T cell” • “chimeric antigen receptor” • “glioblastoma”

Methods-

Data use

- Comparing
  - Changes in bioluminescence of tumor cells from studies
- One-tailed two-sample T-test
  - No Wilcoxon-Mann-Whitney test
  - Statistical significance: $p<.05$

Flow of studies



Xenograft methodology of studies

- Obtain tumor cells

Timeline

- Generate CAR-T

Results:

- Bioluminescence (photons/sec) as tumor volume
- Compare control(s) and treatment

Calculating %Treatment/ΔControl

(Johnson et al., 2015)

$$\%T/C = 100 \times T/\Delta C \text{ if } T \geq 0$$

$$\%\ \text{Regression} = 100 \times T/T_{Initial} \text{ if } T < 0$$

T = Tumor volumes of final day

$T_{initial}$ = Tumor volumes on initial day

$\Delta T = T - T_{initial}$

C = Mean tumor volume of control on final day

ΔC = Mean tumor volume difference between final and initial days

Results

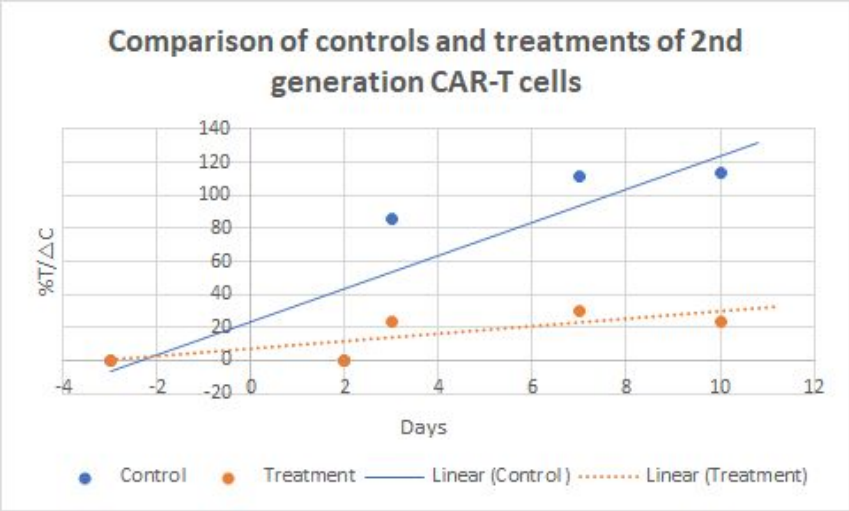
Studies Included

(Wang et al., 2018)

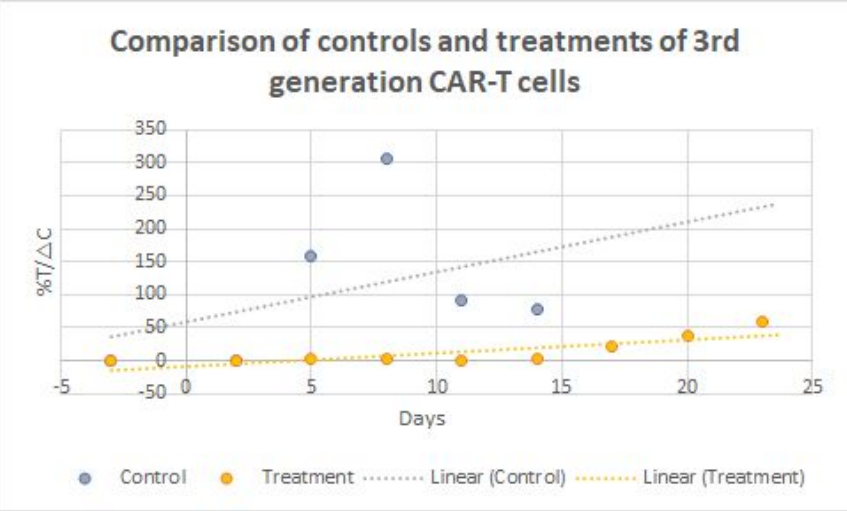
(Miao et al., 2014)

(Johnson et al., 2015)

Results- Individual studies



(Johnson et al., 2015)
Figure 4. Graph comparing the difference in bioluminescence between control and treatment of 2nd generation CAR-T cells



(Miao et al., 2014)
Figure 5. Graph comparing the difference in bioluminescence between control and treatment of 3rd generation CAR-T cells

Results- one-tailed two-sample T-test

2nd Generation
(Day 10 after CAR-T)-

p = 0.00269

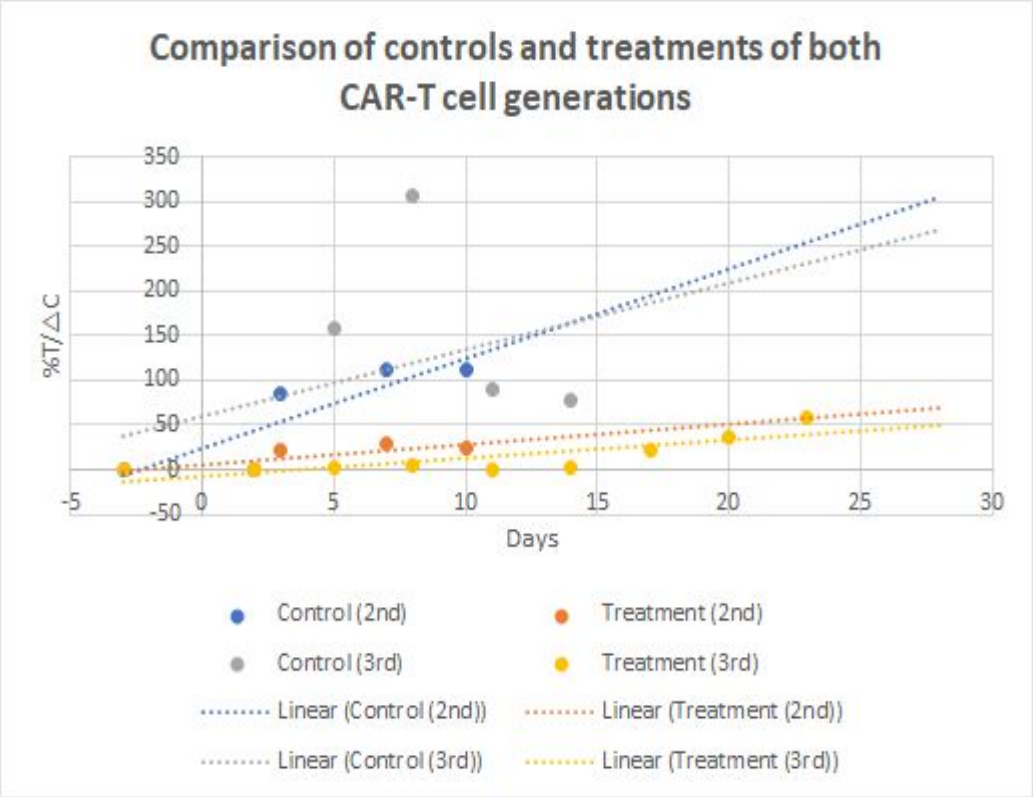
3rd Generation
(Day 11 after CAR-T)-

p = 0.00446

Both statistically significant

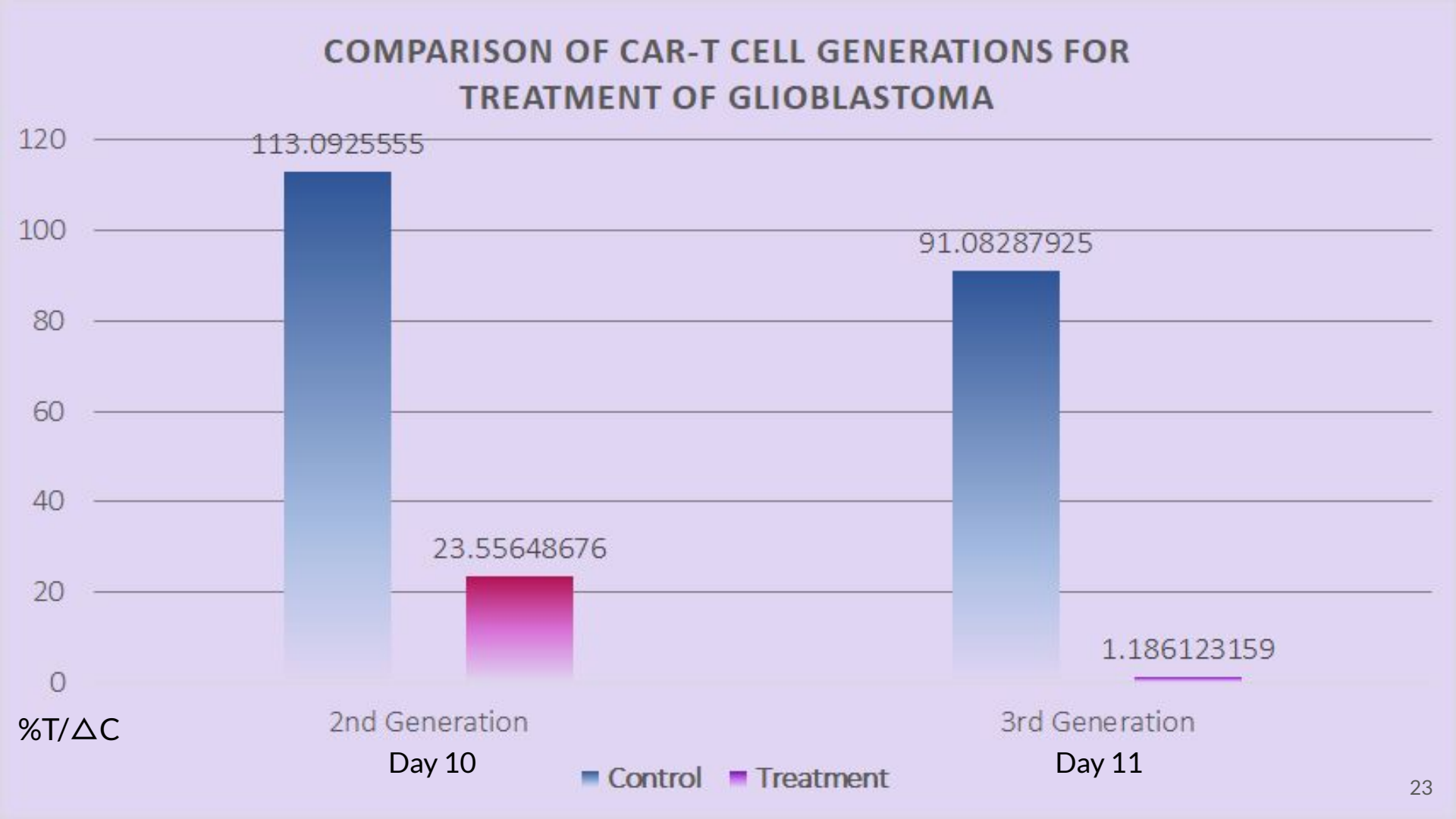
Results

- Less tumor cells in both treatments
- 3rd Generation-
  - Longer survival time



(Johnson et al., 2015; Miao et al., 2014)

Figure 6. Graph comparing the difference in bioluminescence between control (mice with untreated T cells) and the treatment (mice with CAR-T cells) of both generations. This is used as a representation of the difference in tumor cells.


COMPARISON OF CAR-T CELL GENERATIONS FOR TREATMENT OF GLIOBLASTOMA
120
100
80
60
40
20
0
113.0925555
23.55648676
91.08287925
1.186123159
2nd Generation
3rd Generation
%T/△C
Day 10
Day 11
Control
Treatment

$p = 0.00453$

Statistically significant difference between treatment groups

Discussion

- Statistical significance: $p<.05$
- Both treatments effectively targeted tumor cells

Performance:

2nd Generation < 3rd Generation

Limitations

- Studies
  - Representation of clinical performance
  - Off-target toxicity
- Analysis
  - Small sample size
  - Discrepancies between studies

Sources of error

- Differences in:
  - Days administered/ imaged
  - Quantity administered
  - Cell line

Further work

- Investigate confounding variables
- Clinical studies
  - Efficacy
  - Side effects
- Future generations
- Other solid tumors

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