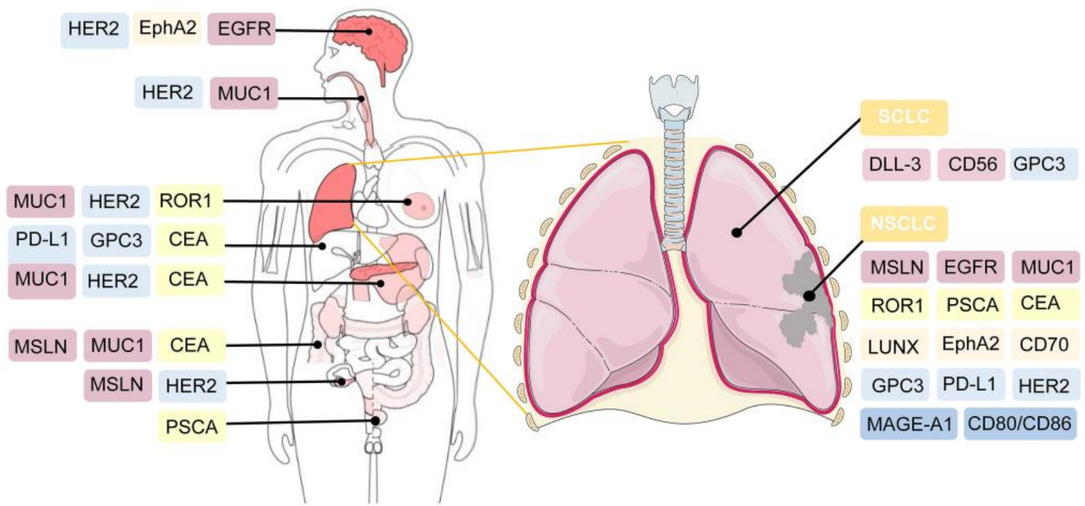
Defeating Non-Small Cell Lung Cancer Using TanCAR-T cells targeting B7-H3 and EGFR: A New Era of Hope

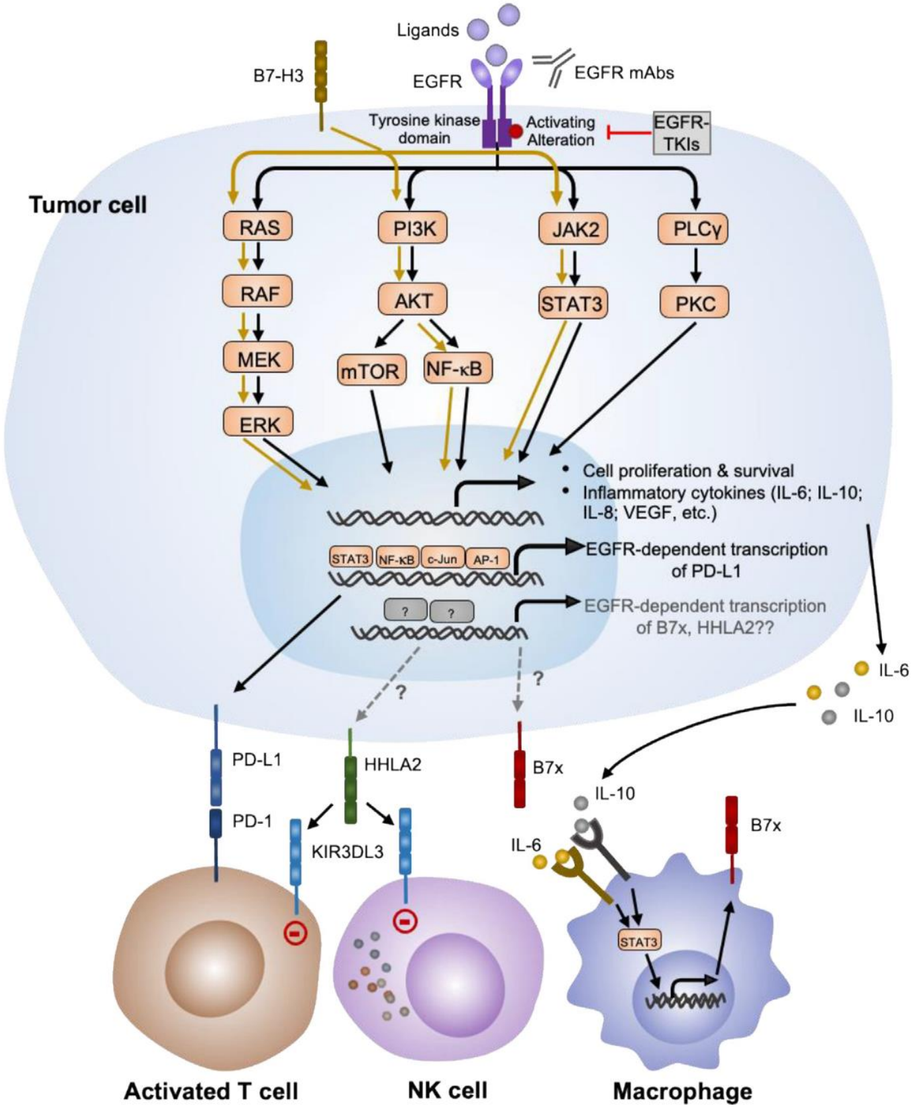
Presented by: Lucia Sileo and Chiara Lollobrigida

1. What do we know?

1.1 NSCLC AND SPECIFIC TARGETS: B7-H3 and EGFR



Chimeric Antigen Receptor T-Cell Therapy in Lung Cancer: Potential and Challenges (*Xiao et al.*)

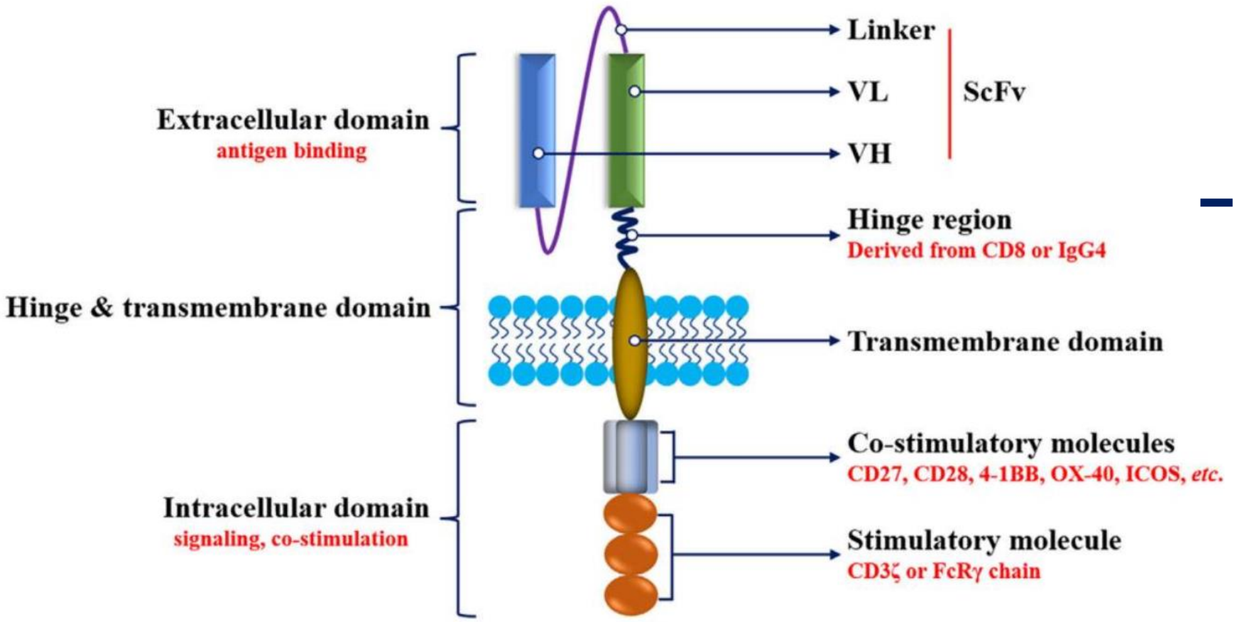


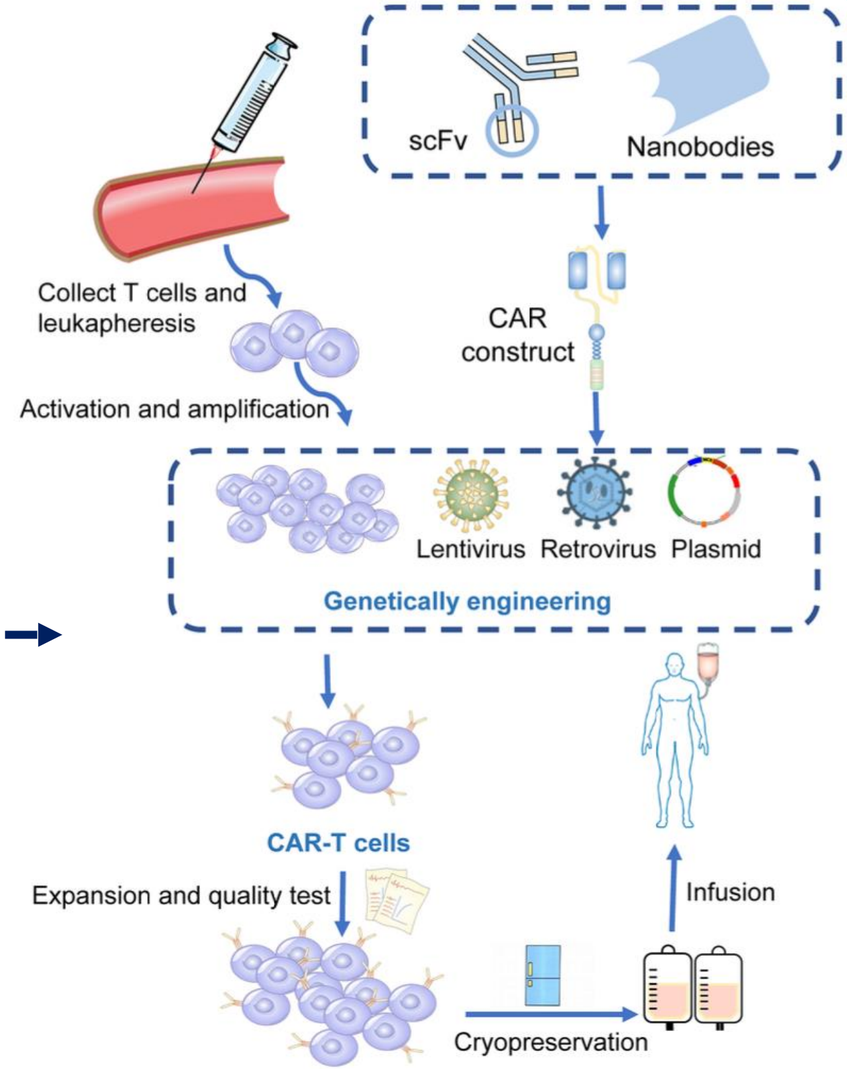
Crosstalk between the B7/CD28 and EGFR pathways: Mechanisms and therapeutic opportunities (*X. Ren et al.*)

1. What do we know?

1.2 CAR-T Therapy

2nd Generation CAR-T cells



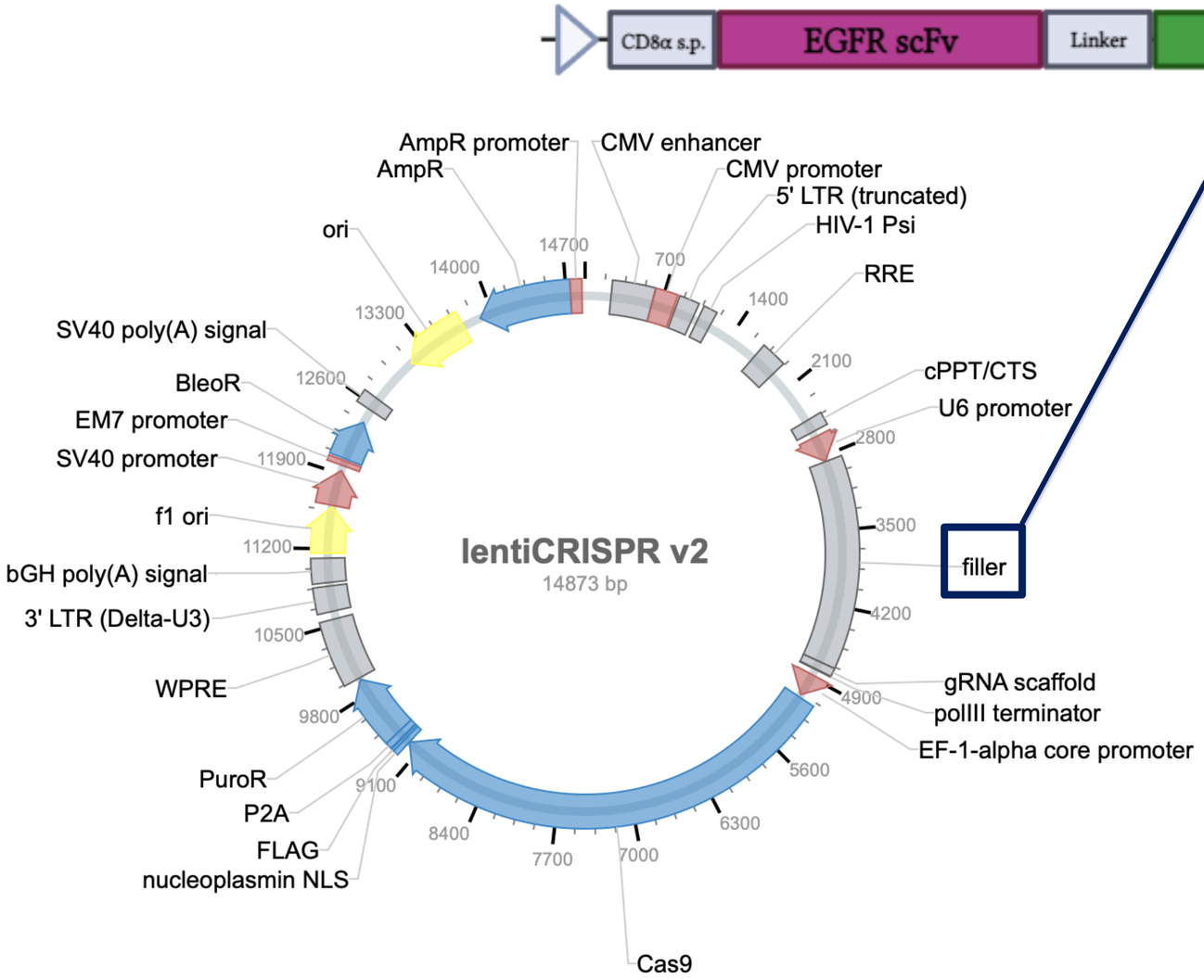


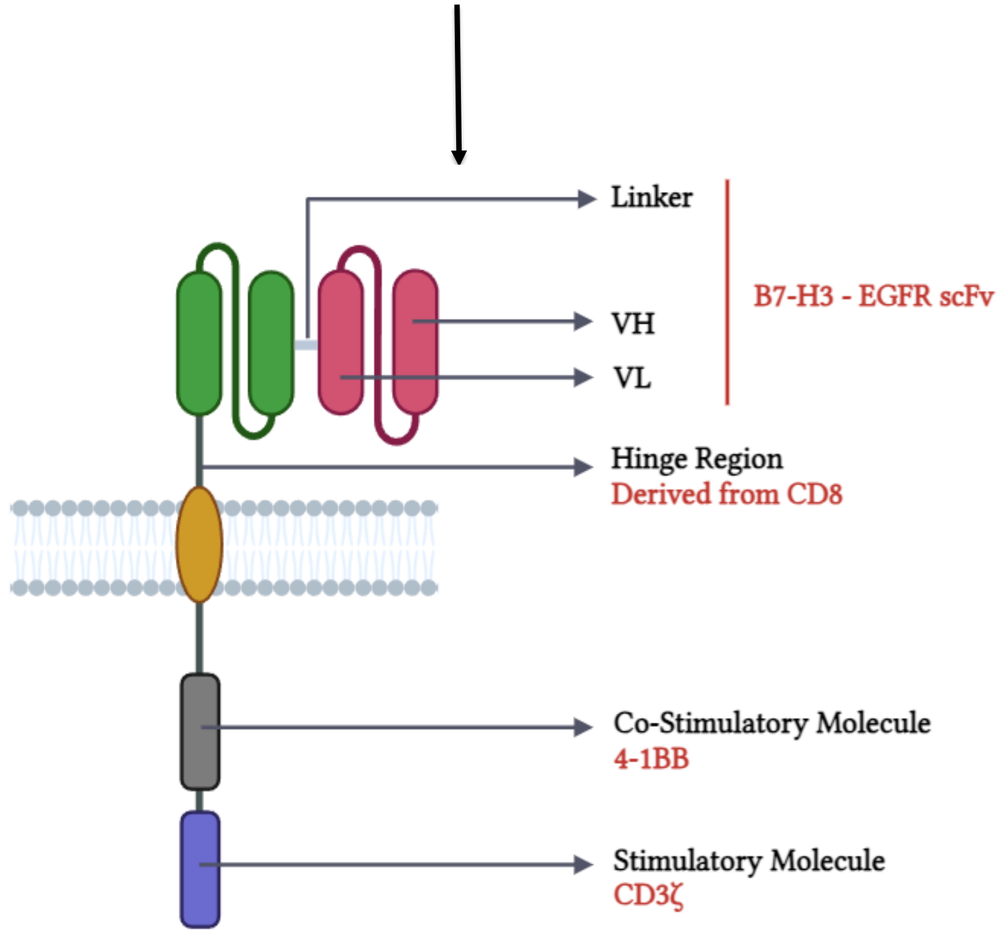
CAR-T cell therapy for lung cancer: Potential and perspective (*Chen et al.*)

Chimeric Antigen Receptor T-Cell Therapy in Lung Cancer: Potential and Challenges (*Xiao et al.*)

2. What do we want to do?

2.1 LentiCRISPRv2 – ENGINEERING STRATEGY





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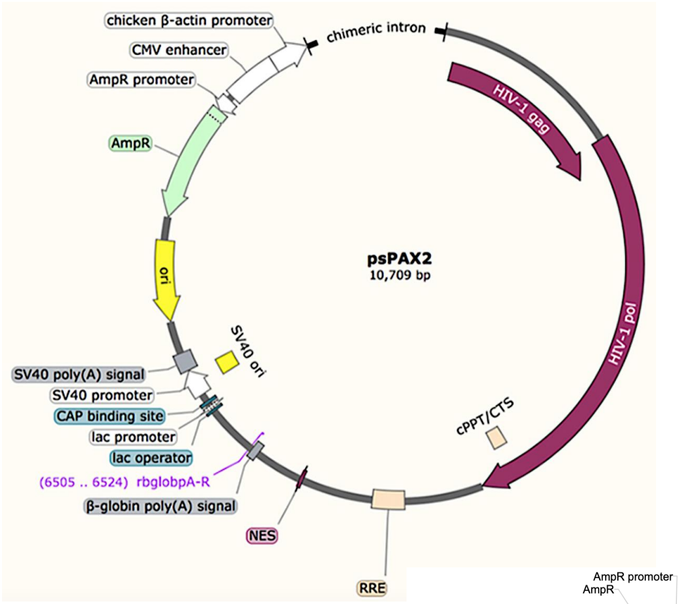
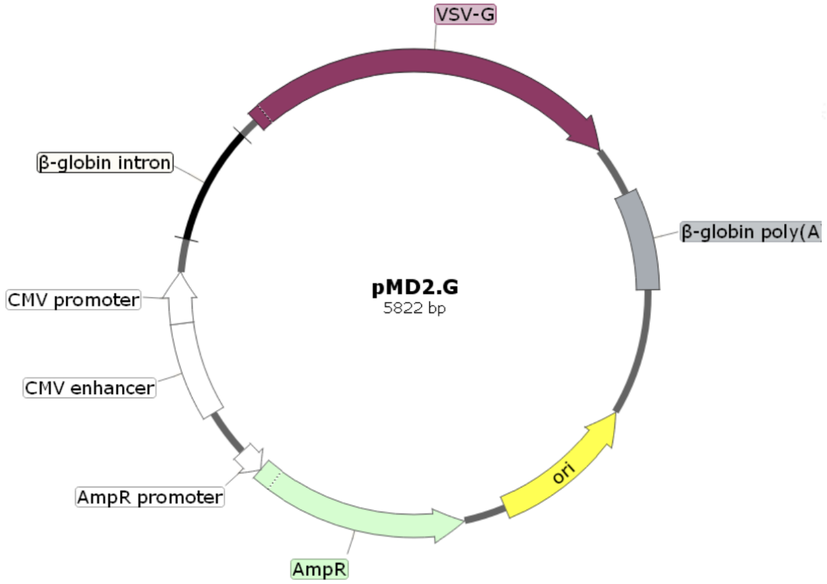
2. What do we want to do?

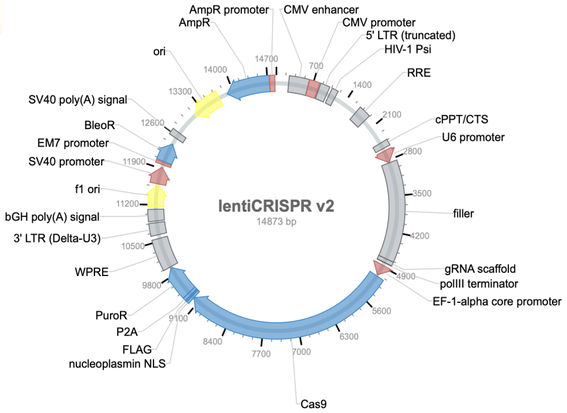
2.2 LentiCRISPRv2 – Vector Production

Packaging Plasmid

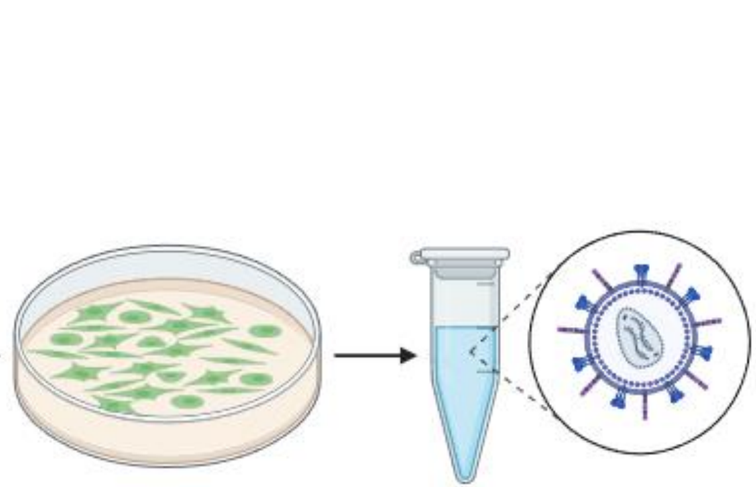


Envelope Plasmid (VSV-G)





Transfer Plasmid



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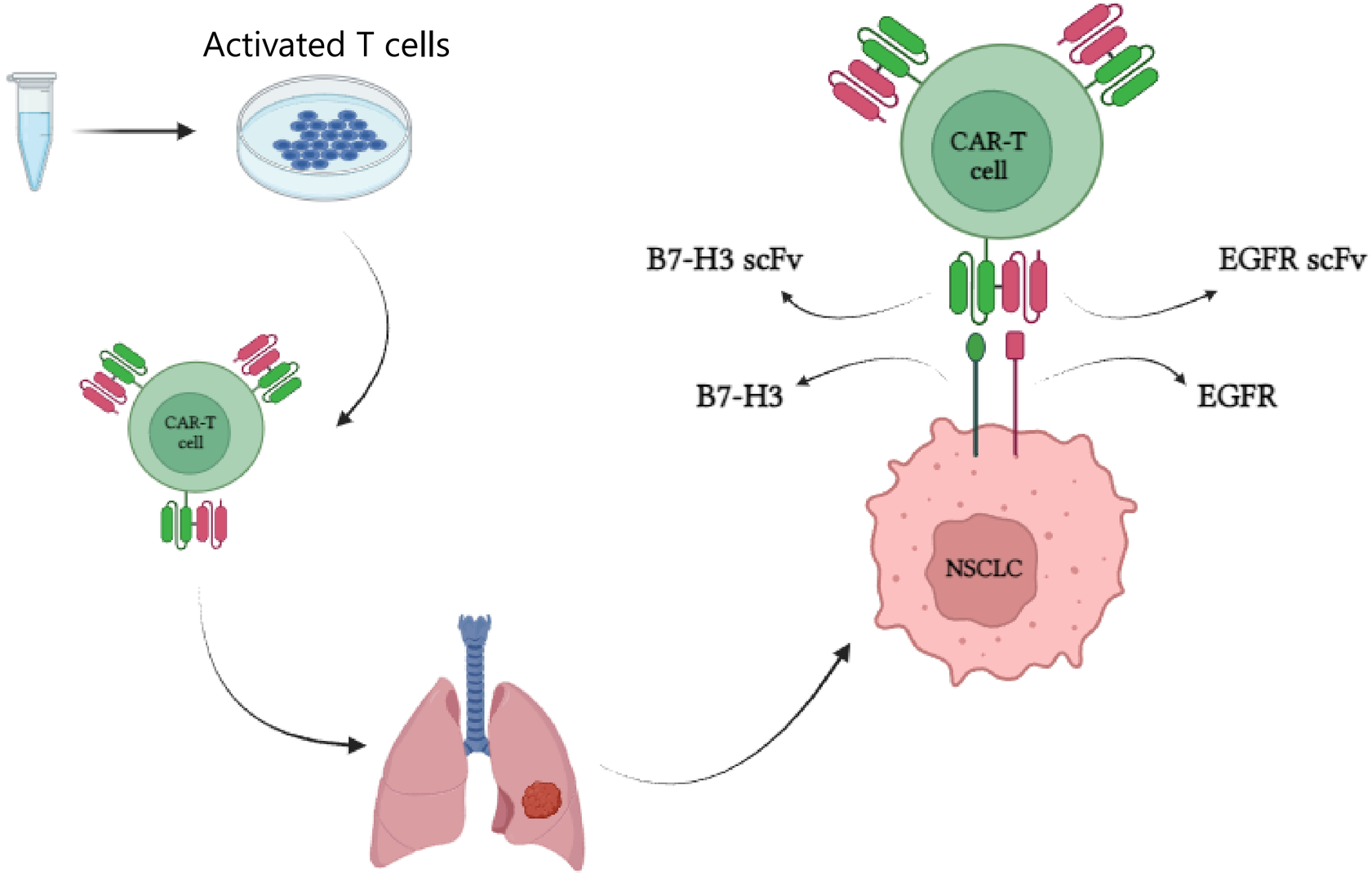
3. How does it work?

3.1 Specific TanCAR-T cells Toxicity Against NSCLCs



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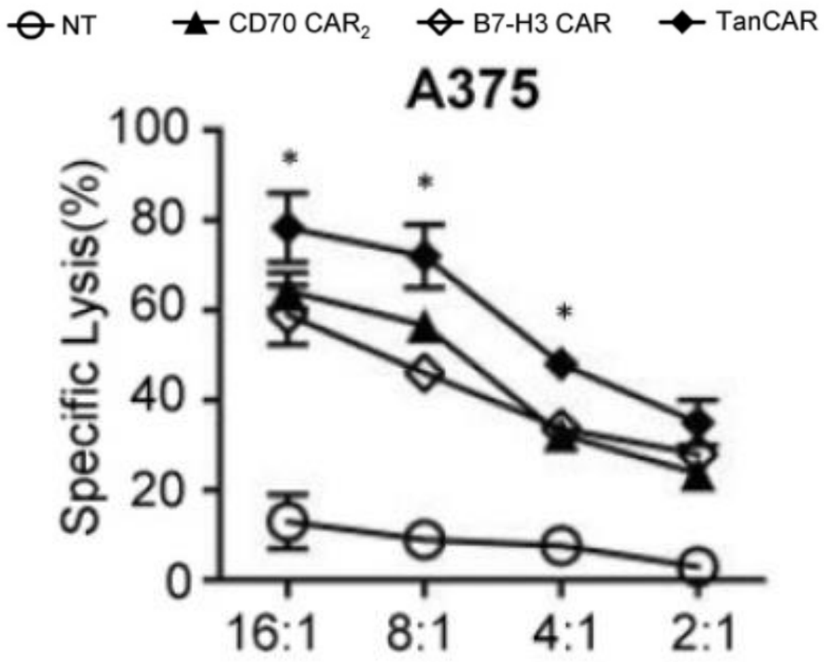
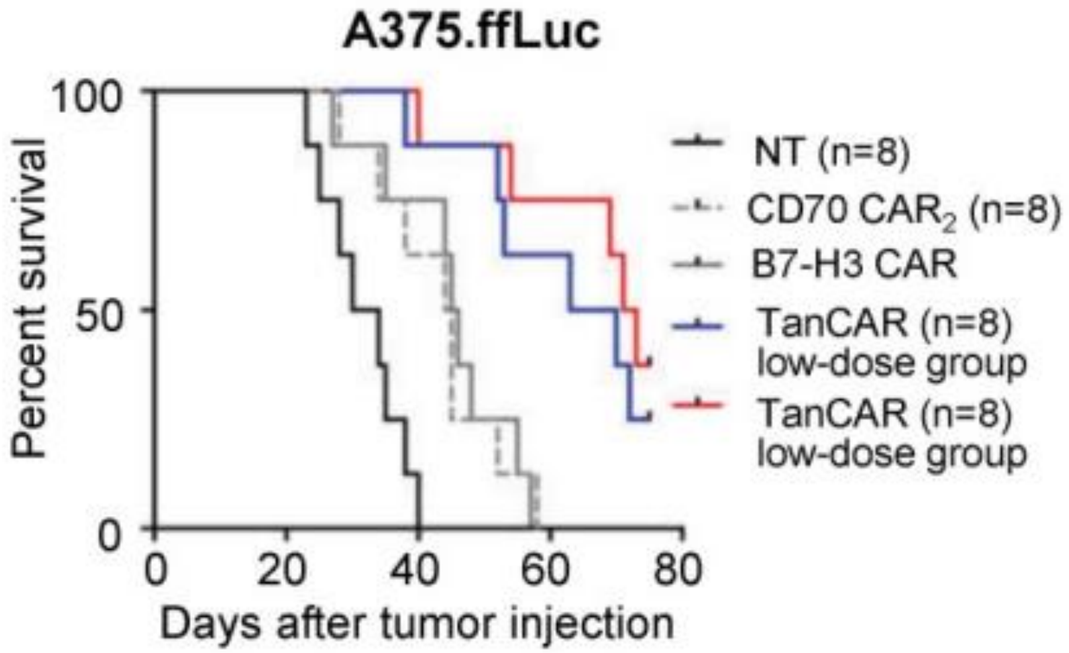
4. Experimental plan

4.1 BACKGROUND

In Vitro Testing



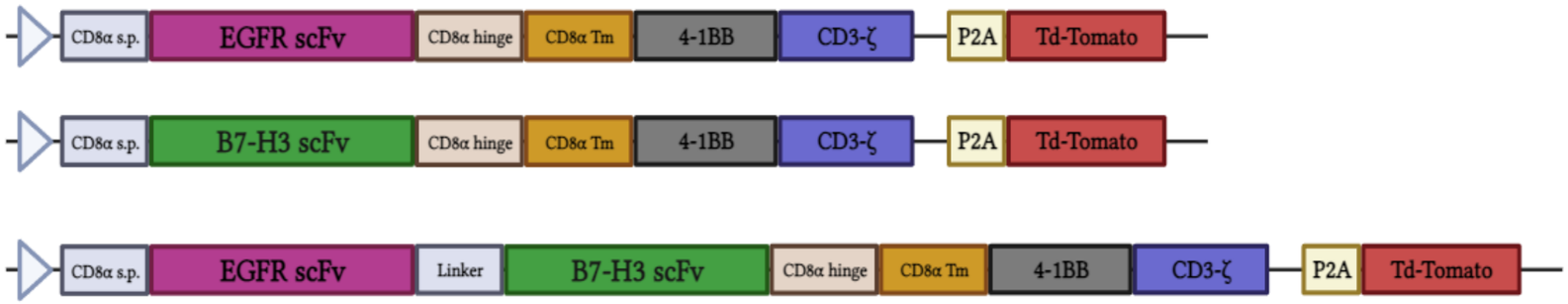
In Vivo Testing

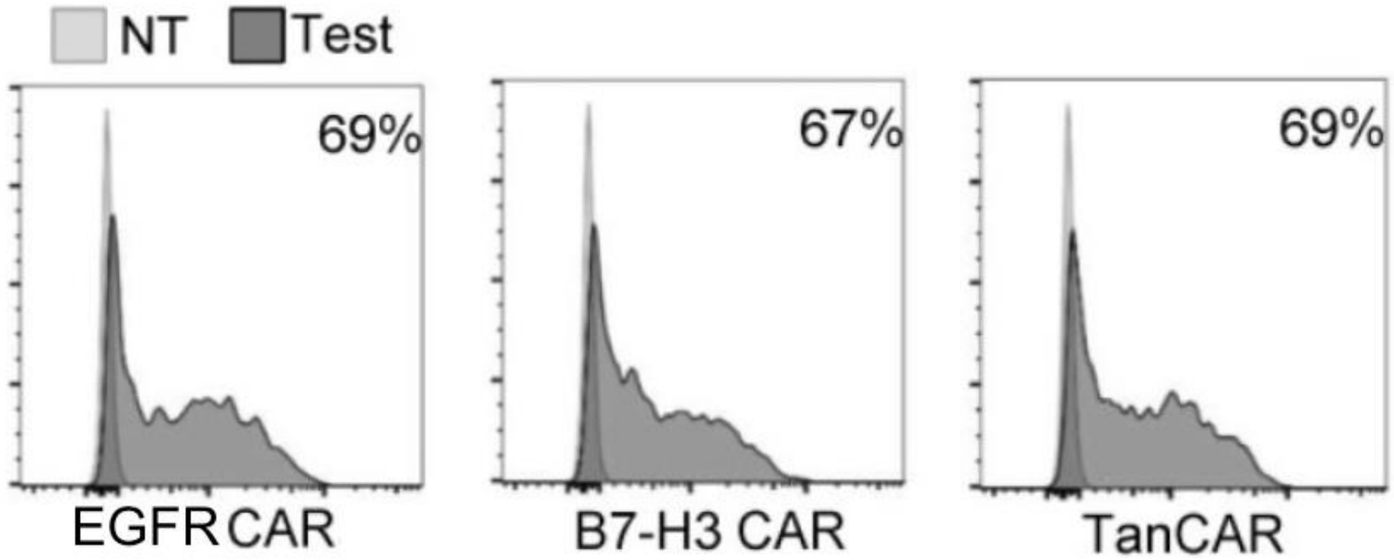


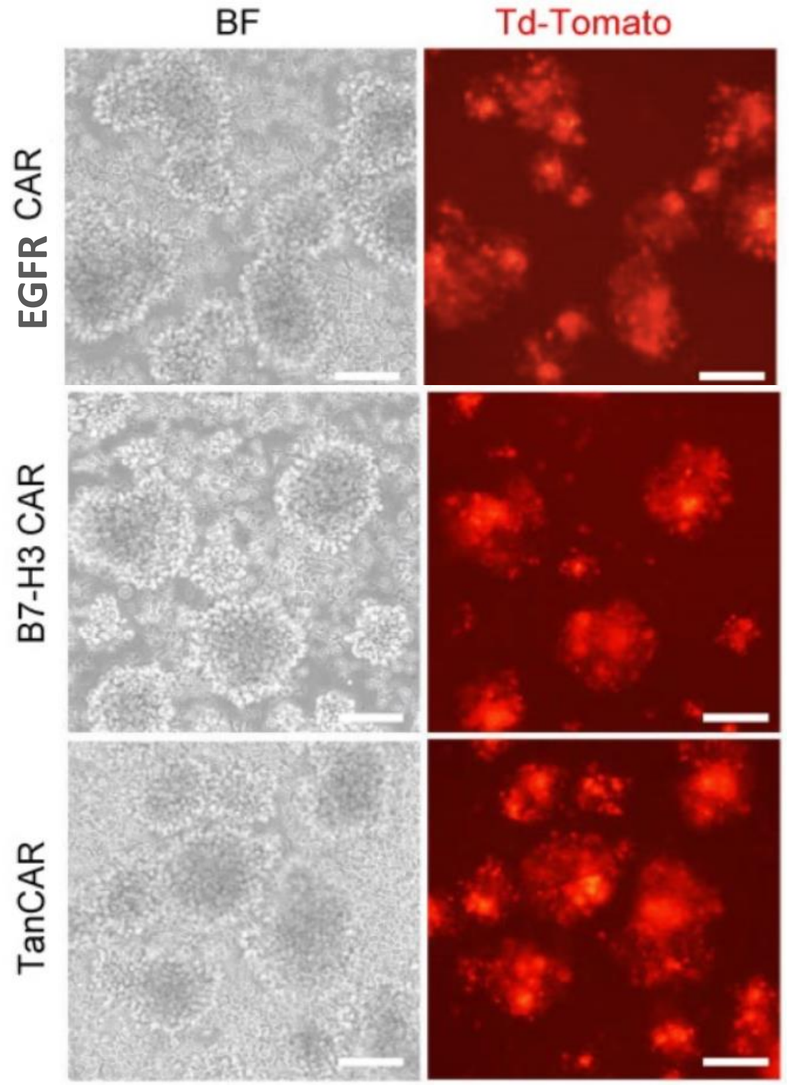
Tandem CAR-T cells targeting CD70 and B7-H3 exhibit potent preclinical activity against multiple solid tumors (Yang et al.)

4. Experimental plan

4.2 In Vitro Testing – Trasduction Efficiency of CAR-T

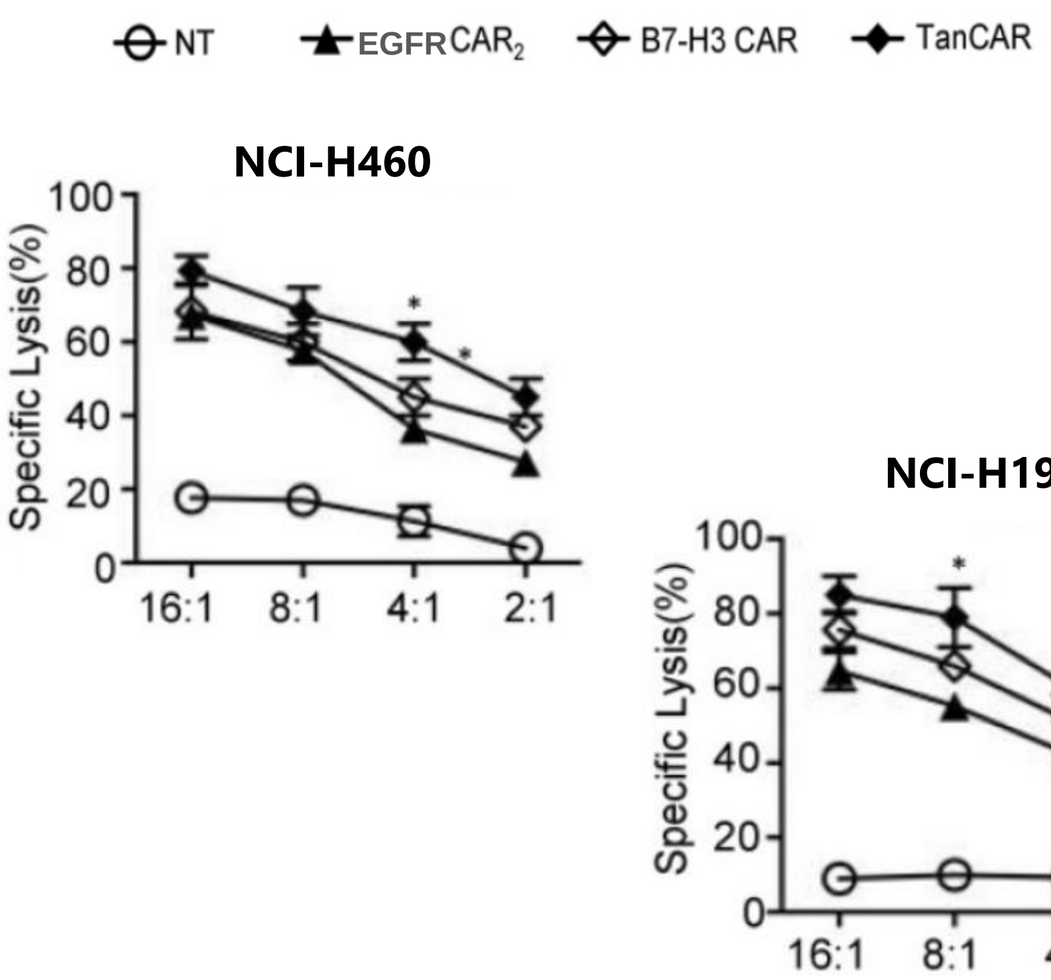


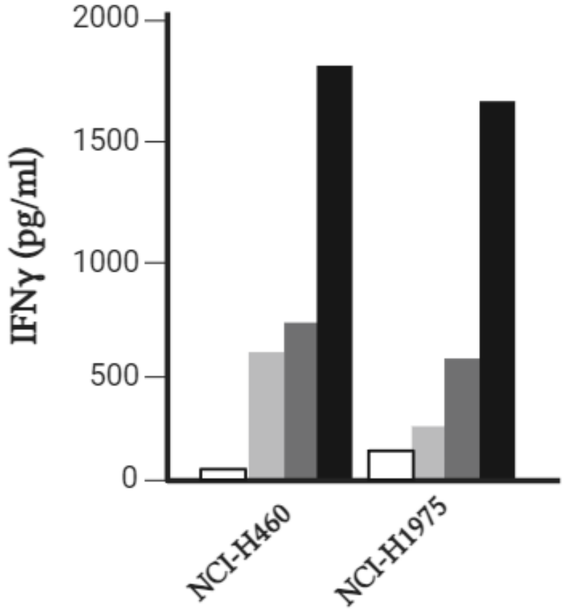


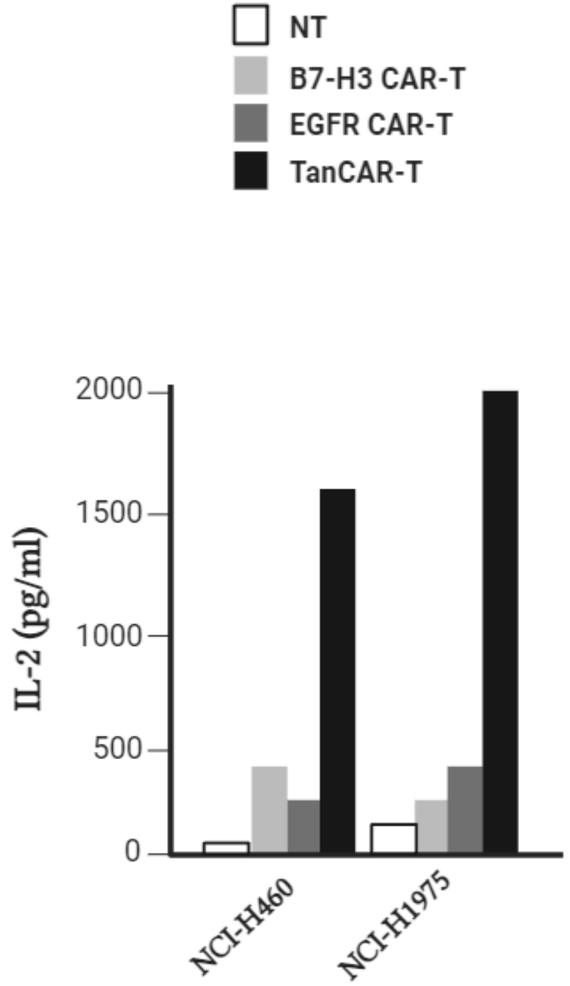


4. Experimental plan

4.3 In Vitro Testing – Anti-Tumor Effect

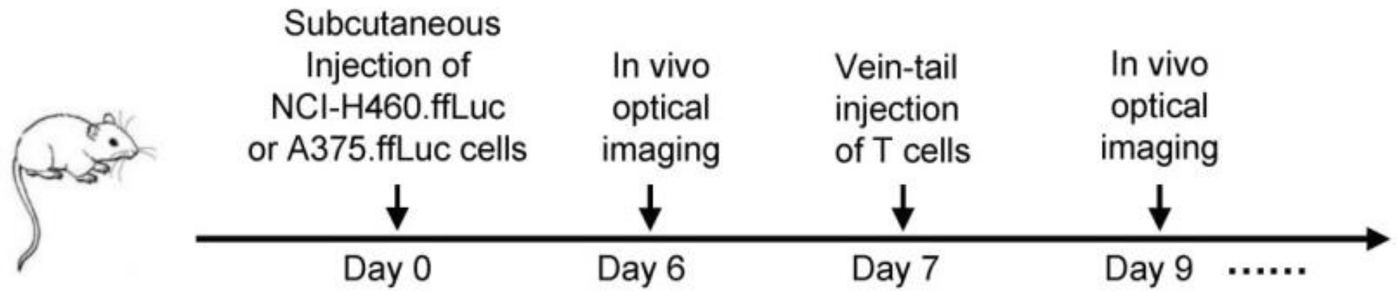


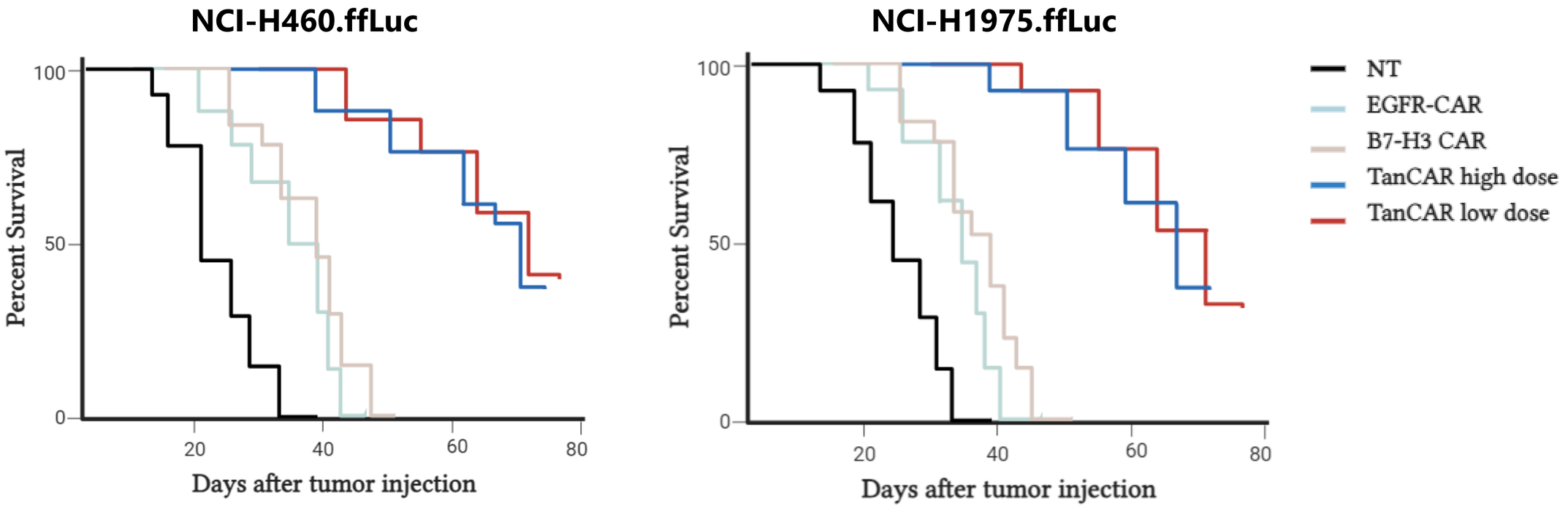




4. Experimental plan

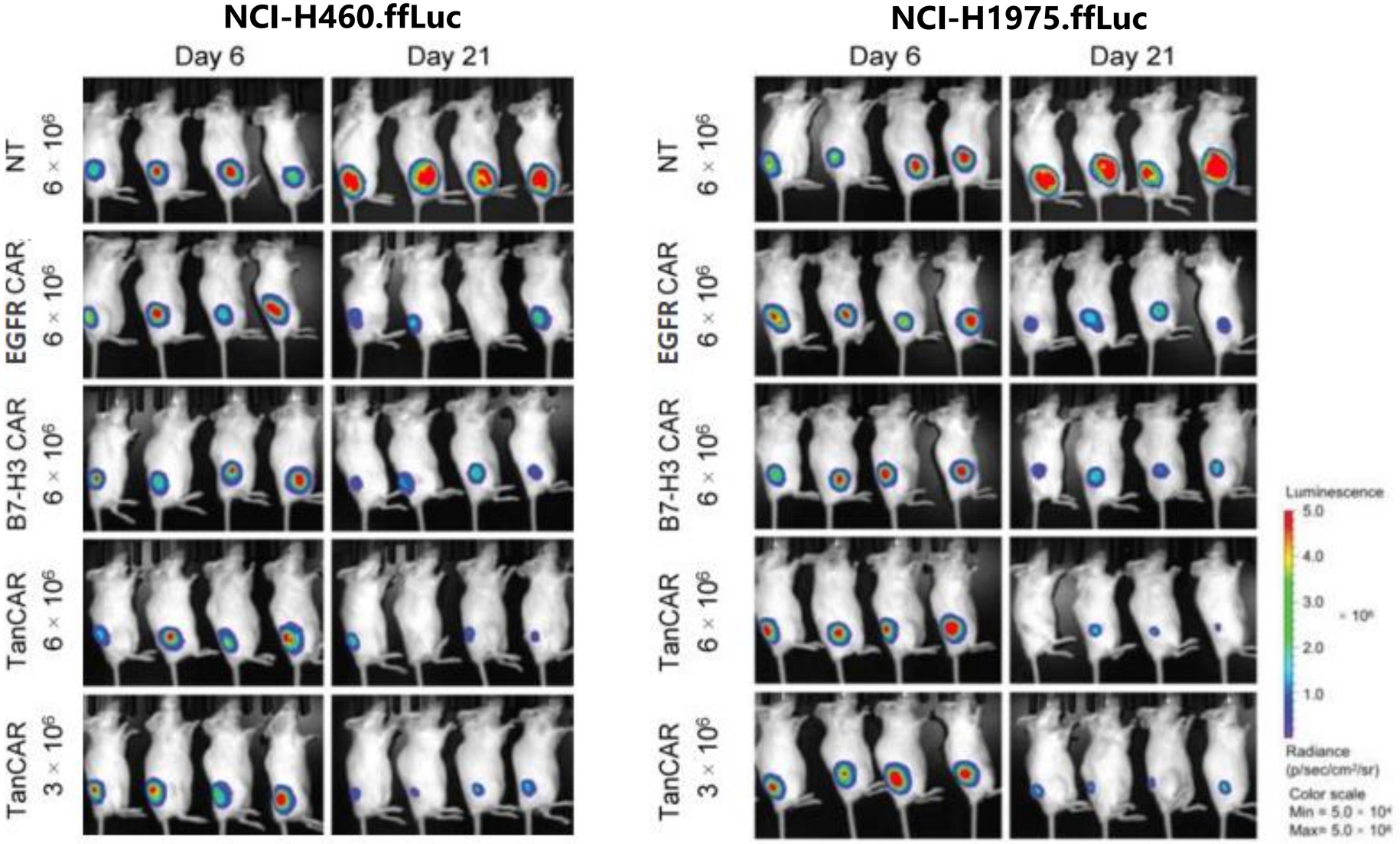
4.3 In Vivo Testing – Anti-Tumor Effect





4. Experimental plan

4.3 In Vivo Testing – Tumor Size Reduction



5. Conclusions

NSCLC

- On most cases, NSCLC expresses B7-H3 and EGFR, proteins involved in similar pathways.

CAR-T Therapy

- We use lentiCRISPRv2 to target both proteins, decreasing the off-target effect of CAR-T cells therapy.

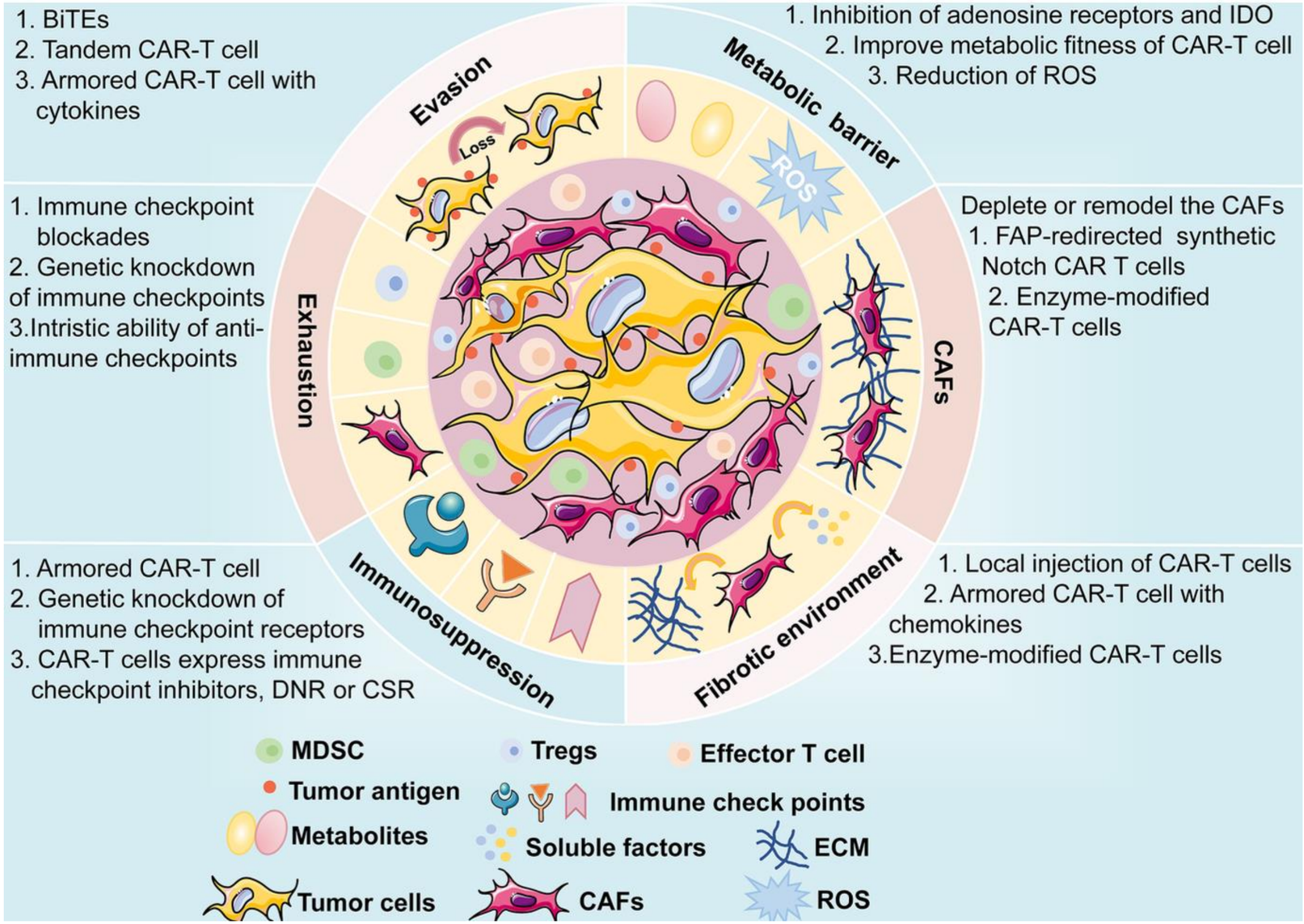
Experimental Plan

- We compare the existing results in the literature and apply them to our cell lines. TanCAR works both *in vitro* and *in vivo*

Anti-tumor Function

- TanCAR-T cells exhibited an enhanced anti-tumor activity and better tumor control in several pre-clinical models

6. Pitfalls VS Solutions



Chimeric Antigen Receptor T-Cell Therapy in Lung Cancer: Potential and Challenges (Xiao et al.)

7. Materials, time and Budget

MATERIALS AND BUDGET

- 40 NSG mice: €5000 + Maintenance (food and water, housing, aseptic conditions, etc...): around €10.000
- Facilities and Work Support:
  1. Enviromental Control: €12.500
  2. Isolation and biosafety: circa €12.500
  3. Imaging Systems: around €110.000
- NSCLC Cell Lines: NCI-H460 - €625; NCI-H1975 - €941; NCI-H460.ffLuc - €1.350; NCI-H1975.ffLuc - €1.350
- HEK-293T cells: €737
- LentiCRISPRv2 + Packaging Plasmid + Envelope Plasmid: €236,52 (2µg each)
- LAB essentials: around €3000
- Employees' Salary: €96.000 (€2.000/month)

TOTAL COST: around €250.000

EXPECTED WORKING TIME → AROUND 12 MONTHS

8. References

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