Module 3: Engineering Cancer Immunotherapy

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BIO467
Scientific
Literature
analysis in
bioengineering

Enhanced intratumoural activity of CAR T cells engineered to produce immunomodulators under photothermal control

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Nature biomedical engineering

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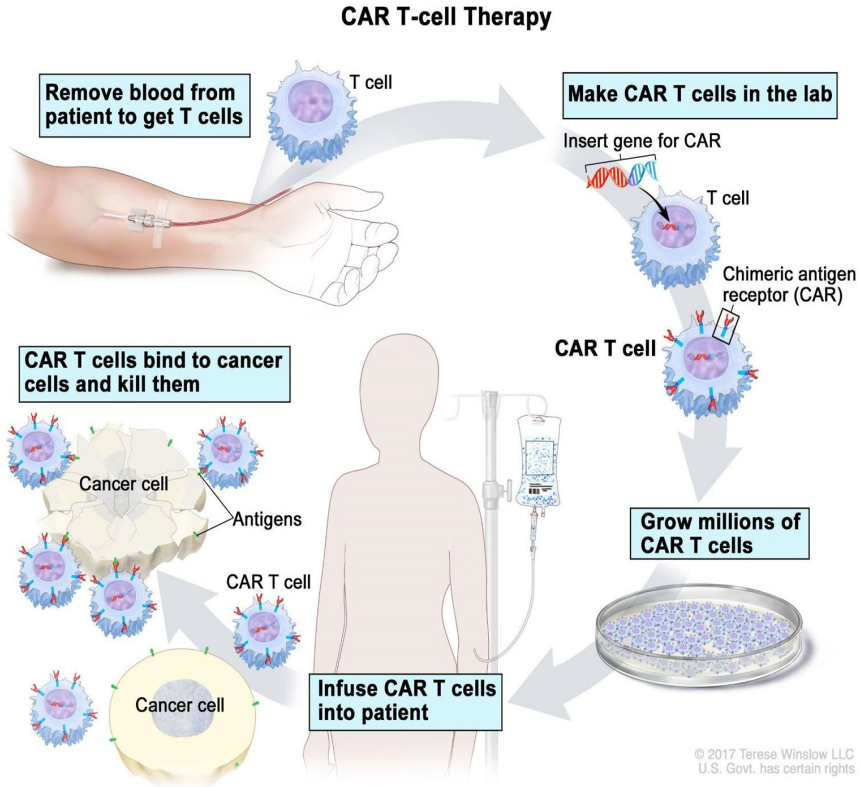
Engineered T cells therapy: Chimeric antigen receptor (CAR) T cells.



1.Thousands of a patient's own T cells are collected in a process similar to blood donation.

2.Introduce genetic modification into the cell.

3.The T cells are reprogrammed ⇒ they produce a special receptors called chimeric antigen receptors or CARs on their surface.

4.These CAR T cells are grown in the lab. Then million of them are infused back to the patient.

5. The new receptor allows them to bind to a specific antigen on the patient's tumor cells and destroy them.

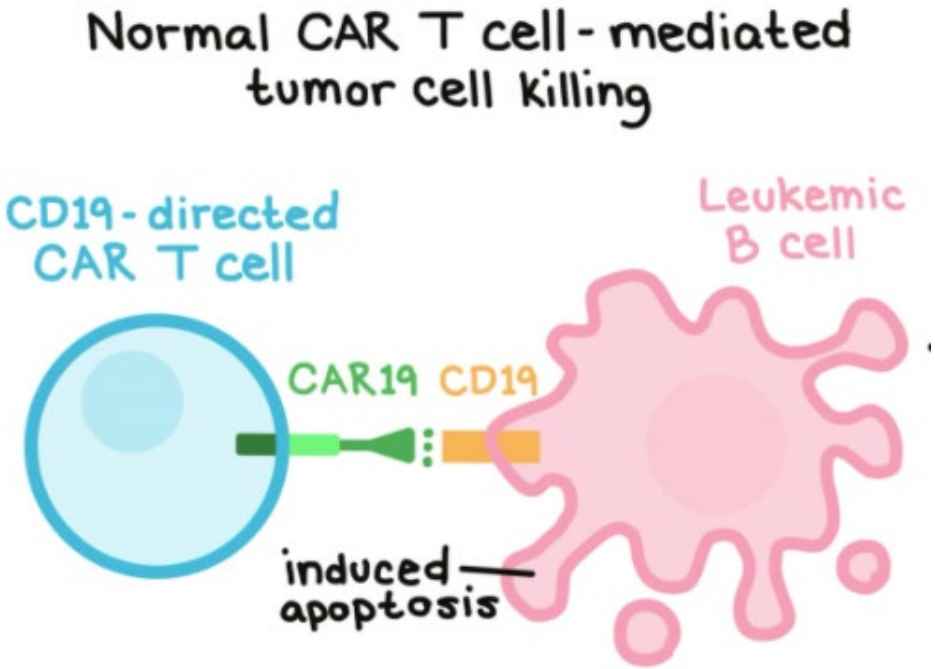
(Definition of CAR T cell Therapy - NCI Dictionary of Cancer Terms - National Cancer Institute. Feb 2, 2011)

Current applications

CAR-T cells have been FDA approved in 2017.

They showed an uncommon success of anti-CD19 CAR-T cell therapy against B-cell malignancies (Type of cancer affecting blood cells).

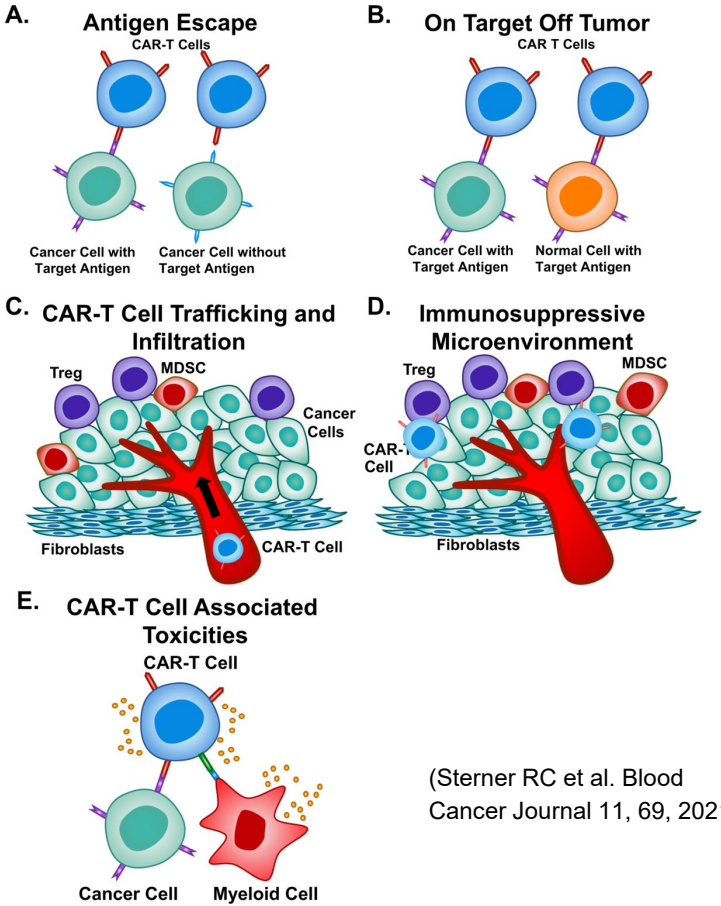
Liquid Tumor



(Hitchings L. “When CAR T cell therapy crashes”. ACIT. Oct 24, 2018)

Limitations in Solid tumors

- **Excessive** level of complexity.
- **Rarity** of tumor antigen. (Detection Problem).
- **Inefficient** persistence & expansion of adoptively transferred T cells.
- **Immunosuppression** by the tumor environment.



(Sterner RC et al. Blood Cancer Journal 11, 69, 2021)

Current methods promoting intratumoral activity of modified T cells & their drawbacks.

- Administration of **immunostimulatory agents**. Exp: cytokines.
- **Checkpoint blockade inhibitor** antibodies.
- **Bispecific** T cell engagers.

- **Lack of specificity**. Exp: activate both engineered and endogenous immune cells !
- **Toxicity** in health tissue.
- **Limitation** of the tolerable doses.

Develop targeting and local enhancement of T-cell function (CAR) at tumor disease sites.

- Use of biomaterials:
  - Biopolymer scaffolds loaded with tumor-specific T cells.
  - Biopolymer scaffolds loaded with immunostimulatory adjuvants.
- Genetic modification:
  - **Sensing and response biocircuits:** Conditional activation in the presence of specific input signals. Exp : Hypoxia, heat, etc

Development of a technology for photo-thermal control of T cell therapies.

Engineer T cells able to react to heat :

1) Synthetic gene switches ⇒ Trigger the expression of transgenes in response to (40-42°C).
2) Designing thermal constructs to produce 1) IL-15 superagonist & 2) (NKG2DL) BiTE.

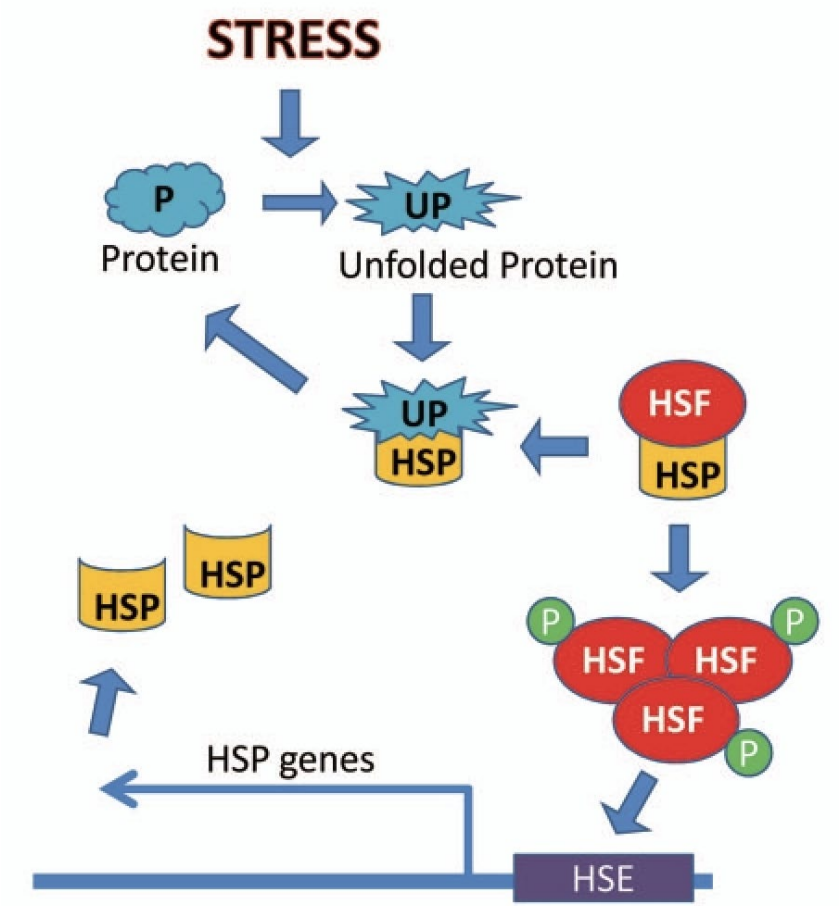
Typical clinical use of heat treatment (overview):

- Sensitize cancer cells to chemotherapy (40-42°C)
- Removal of isolated metastatic nodules (>50°C)

Technology used to target the tumor:

- Laser interstitial thermal therapy (LITT)

Cellular response to Hyperthermia:



(Krivoruchko A et al. Oxidative Medicine and Cellular Longevity 3, 3, 2010)

Principle:

- Various stresses can lead to unfolding of proteins, calling heat shock proteins (HSPs) into action to aid refolding.
- As a consequence, HSPs dissociate from association with the heat shock transcription factor (HSF).
- The freed HSF becomes activated through trimerization and binds to HSE and mediates the upregulation of hsp genes.
- This results in the proliferation HSPs in the cell.

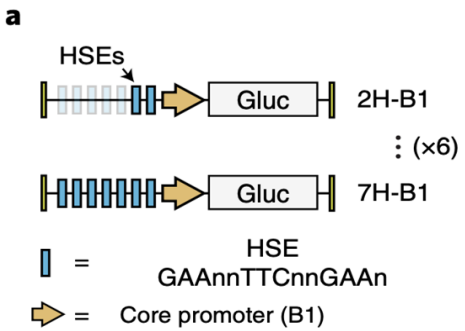
Problem:

- The response of endogenous HSP genes is selective but not specific for heat as their promoters contains additional regulatory elements (for example, hypoxia response elements or metal-responsive elements).

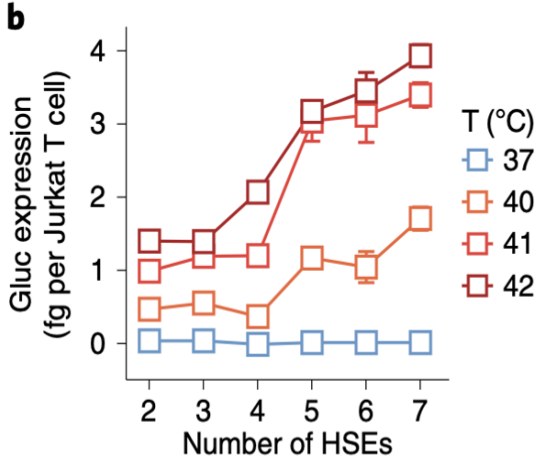
Solution:

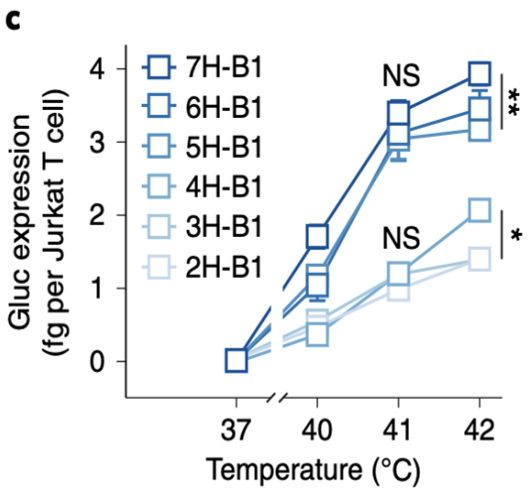
- Build synthetic gene switches that are activated by heat but not by other sources of stress.

Engineering thermal-specific gene switches:



- Six candidate constructs of thermal gene-switch consisting of two to seven HSEs upstream of the *HSPB1* core promoter.
- *HSPB1* core promoter was selected as its parent genes were upregulated by more than 20-fold at 42 °C in primary murine T cells.





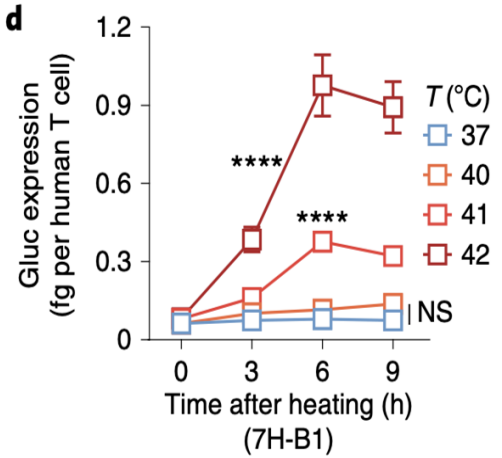
Thermal response was tested in primary human T cells.

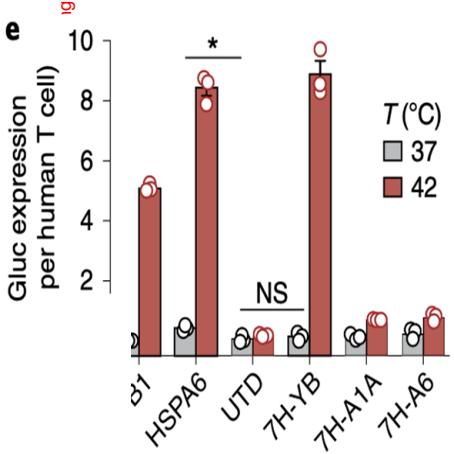
- To quantify responses of the thermal switches, transduced T cells were heated to 3–5°C above body temperature.

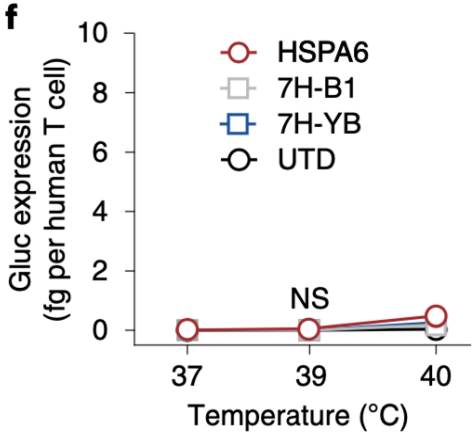
Results :

- Increased expression of the reporter *Gaussia* luciferase (Gluc) as the temperature and number of HSEs increased.

Thermal response :







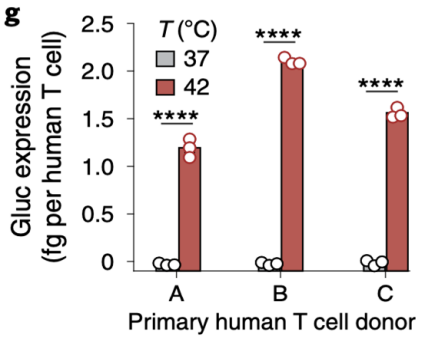
Thermal response in primary human T cells with 7H-B1:

- T cells with the 7H-B1 construct had peak thermal activity approximately 6h after heating at temperatures above 40°C.

Dependency on the core-promoter sequence in primary human T cells thermal responses :

- Different core-promoters identified in the qPCR screen were compared.
- The 7H-YB construct resulted in the highest increase in Gluc reporter levels after 30 min at 42 °C, corresponding to a ~60-fold increase in activity.
- Negligible activation was observed at temperatures of 37–40°C for 24h, which correspond to fever range.

Thermal donor-dependency and specificity:

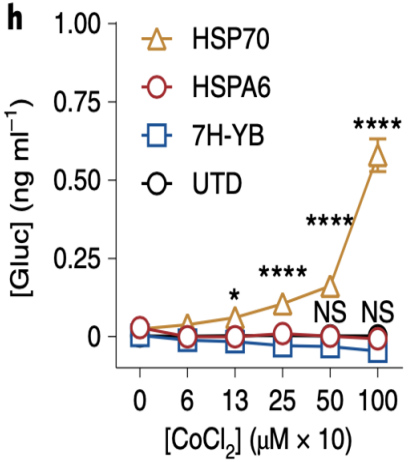


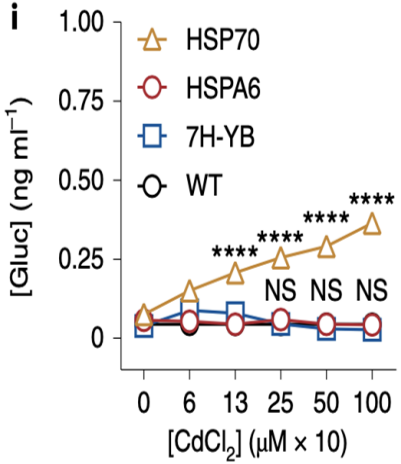
7H-YB thermal activation in T cells derived from three separate donors.

- Confirmation of lack of donor-dependency.

Thermal specificity tested using hypoxia and heavy-metal toxicity as two representative non-thermal stresses.

- 7H-YB compared against endogenous *HSPA6* or *HSP70* promoters, which are highly stress-inducible.
- Transduced primary human T cells were incubated with the hypoxia-mimetic agent (CoCl2), as well as the heavy-metal complex agent (cadmium chloride CdCl2).





Results :

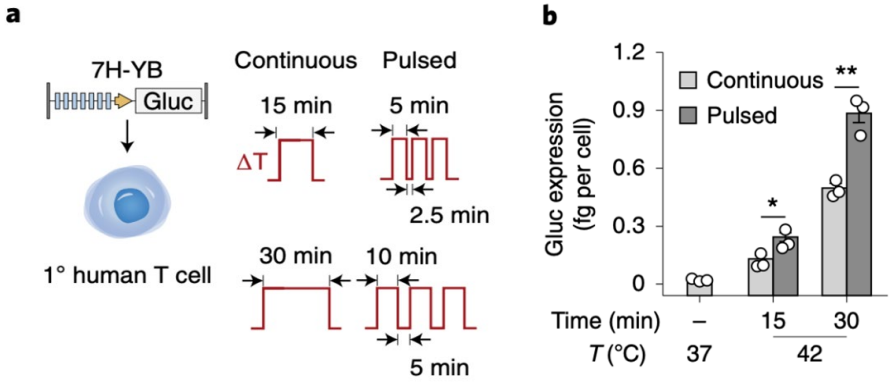
- *HSP70* or *HSPA6* promoter showed dose-dependent activation by hypoxia and cadmium toxicity.
- 7H-YB was not activated and remained similar to untransduced controls.

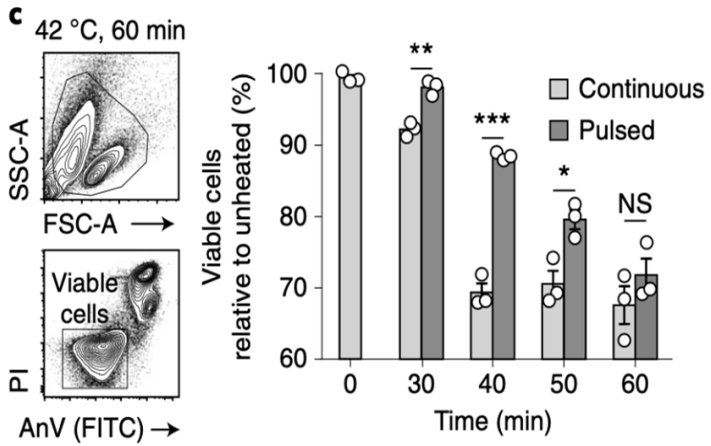
Does Primary T cells maintain key functions after thermal treatment ?

- **In thermal medicine, heating at temperatures greater than 50°C is used to locally ablate tissue by inducing tumour-cell apoptosis, but at temperatures below 45°C, exposure to mild hyperthermia is well-tolerated by cells and tissues due to induction of stress-response pathways including HSPs.**

- **Next step consist on the identification of thermal delivery profiles that would be well-tolerated by primary T cells without affecting key functions including proliferation, migration and cytotoxicity.**

Primary T cells maintain key functions after thermal treatment





Comparison of two heat treatments in primary human T cells transduced with the 7H-YB Gluc vector :

- Unfractionated continuous heating of 15 or 30 min.
- Pulsed heat treatments at 67% duty cycles consisting of three discrete thermal pulses separated by intervening rest periods at 37°C

Results:

- Pulsed heat treatments resulted in up to 87% higher Gluc expression compared to unheated treatment.

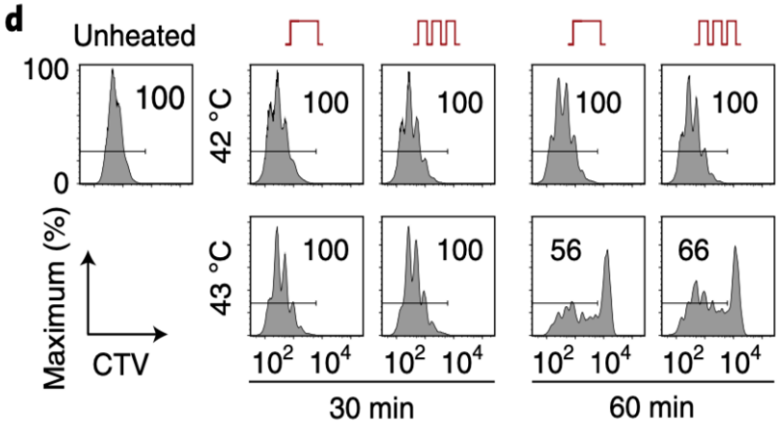
T cells viability :

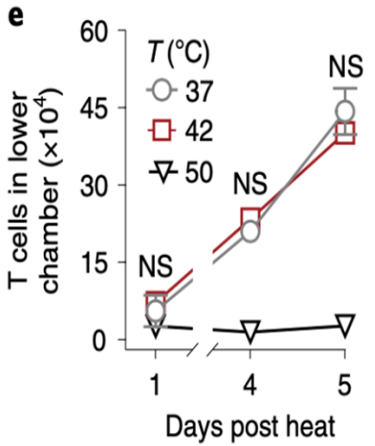
- Quantification of death (propidium iodide, PI) and apoptotic (annexin V, AnV) markers

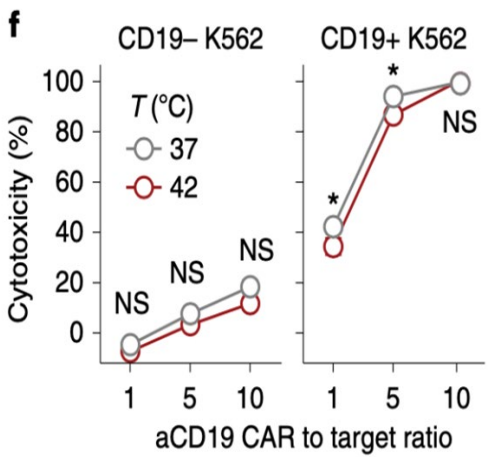
Results:

- Significant improvements for primary T cells that received pulsed treatments at a 67% duty cycle for durations of 30–60 min.

Primary T cells maintain key functions after thermal







T cell proliferation assays :

- Incubation with CD3/28 beads (in order to activate T cells).

Results:

- T cells was unaffected by both continuous and pulsed heating for 30 min at 42 °C or 43 °C, while samples that were heated for 60 min resulted in reduced T cell proliferation .

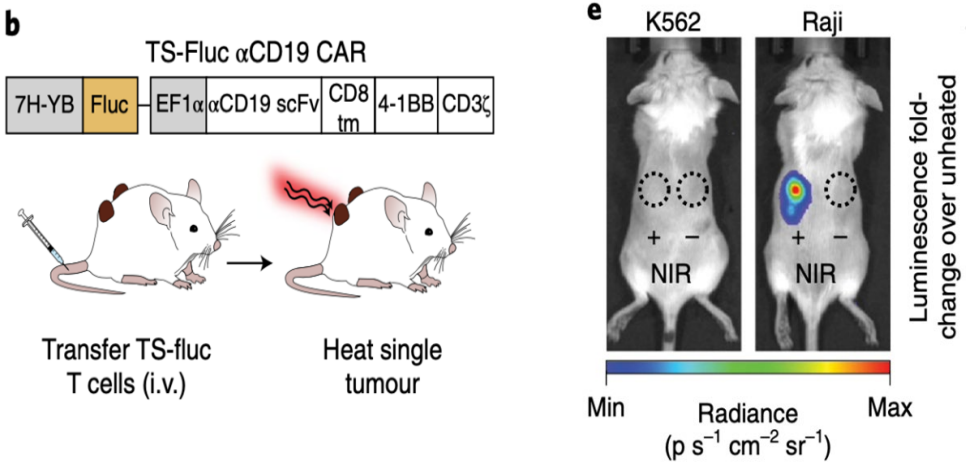
T cell migration, cytotoxicity :

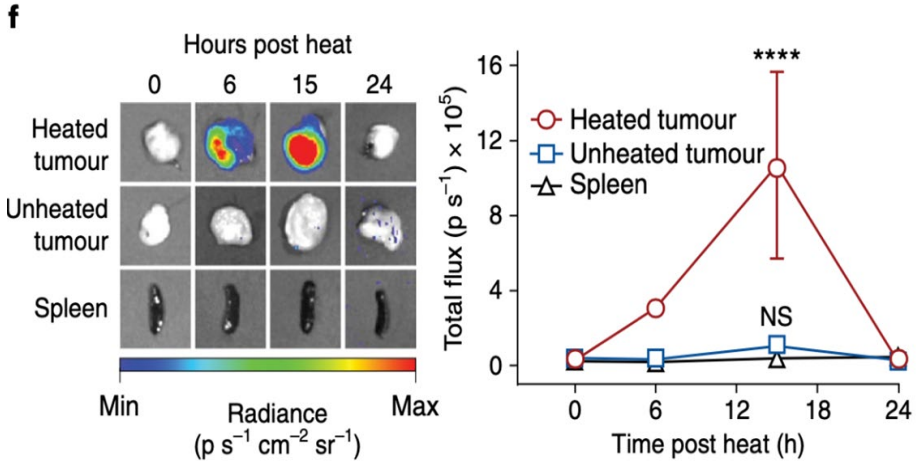
- A lower wells containing a chemokine (CXCL12) to probe T cells migration .
- EF1α promoter in T cells to allow quantification of cell death by loss of luminescence.

Results:

- Heat treatments (42°C for 30min) did not significantly affect T cell migration into lower wells containing the chemokine, whereas T cells heated to 50°C were affected .
- No significant difference in cytotoxicity was observed.

Photothermal activation of T cells in vivo





Spatially targeted activation of T cells by photothermal heating :

- Use of plasmonic gold nanorods (AuNRs) as antennas to convert incident near-infrared (NIR) light (~650–900nm) into heat.
- T cells were inoculated in NSG mice with bilateral flank CD19+ Raji tumours or CD19− K562 tumours.

Results:

- After 20 min heat treatments, luminescence increased by more than 30-fold in Raji tumours that received NIR light compared with unheated or K562 tumours.

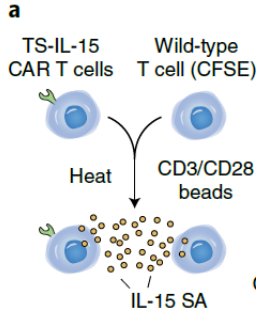
Migration of heat activated T cells out of tumours could result in off-target expression of transgenes :

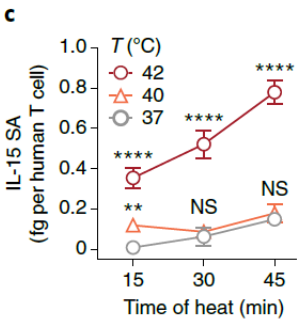
- Single tumour site was heated in order to quantify Fluc activity in the distal tumour and the spleen.

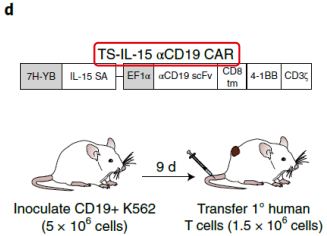
Results:

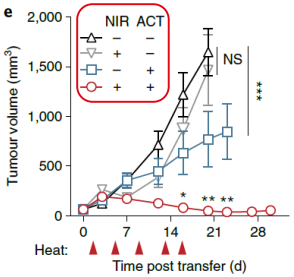
- Luminescence in heated tumours increased within 15h after heating, unheated tumours and spleens remained at baseline levels.
- Indicating that T cells was spatially confined to the heated site.

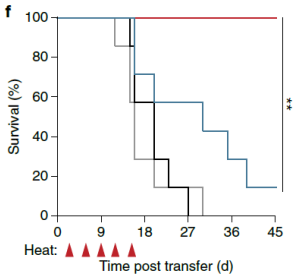
Photothermal control of IL -15 superagonist(SA) enhances adoptive cell transfer(ACT)











The thermal effect of heat-triggered secretion of IL-15 SA

Results:

- TS-IL-15 αCD19 T cells can produce active levels of IL-15 SA following a single thermal treatment
- IL-15 SA levels increased with the duration and temperature of thermal treatment

The therapeutic effect of thermal targeting

- Transferred TS-IL-15 αCD19 CAR T cells into NSG (immunodeficient) mice bearing tumours
- Controlled the two conditions of NIR heating and ACT

Results:

- Group with both ACT and NIR markedly reduced tumour burden and improved survival rate
- **Similar** results were achieved in immunocompetent mice

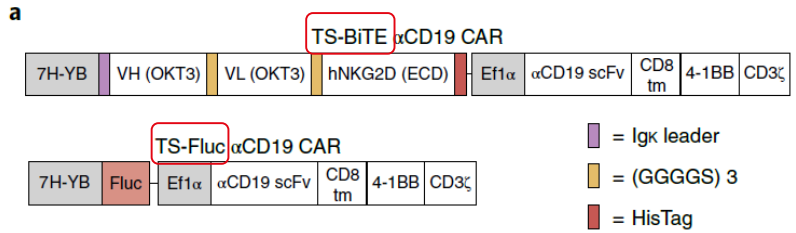
Conclusion:

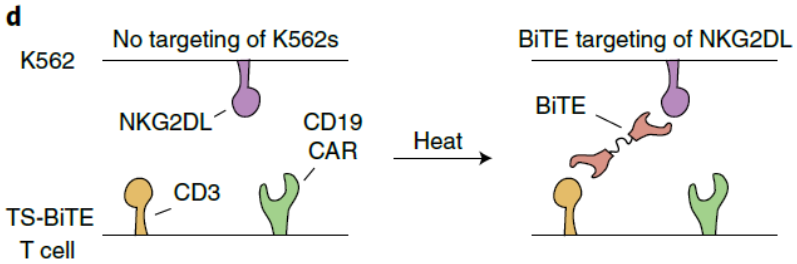
- Photothermal control of IL-15 SA production by engineered T cells significantly improves tumour control

TS-BiTE αHER2 CAR T cells mitigate antigen escape

Heterogeneous expression of antigens can lead to **tumour escape** from CAR T cells that are directed against a single antigen

➔ Therefore, to explore whether heat-triggered expression of a BiTE(Bi-specific T-cell engager) targeting NKG2D ligands could mitigate antigen escape

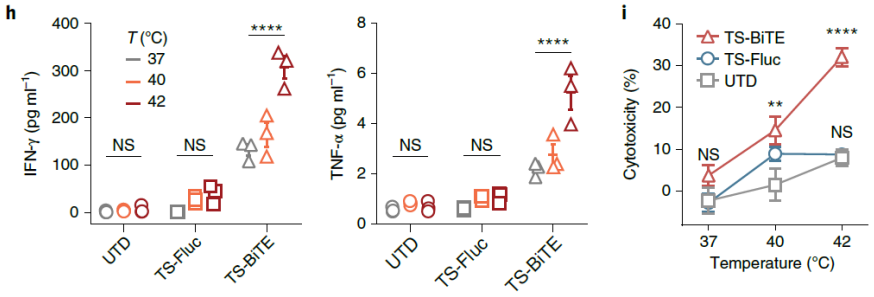




First: To target antigen-negative tumour cells

- Schematic of TS-BiTE and TS-Fluc thermal switches containing heat-triggered BiTE or Fluc reporters
- Schematic depicting BiTE-mediated targeting of K562 target cells lacking the CAR target antigen via BiTE binding to NKG2DL and CD3
- **A way to deal with antigen-negative tumour cells**

TS-BiTE αHER2 CAR T cells mitigate antigen escape



To quantify cytotoxicity from heat-triggered expression of BiTEs

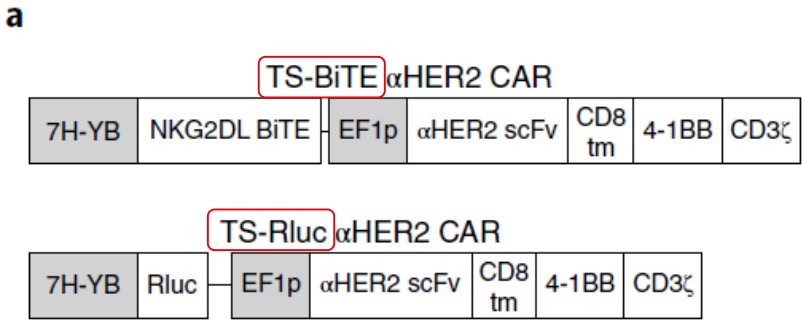
Results:

- TS-BiTE CAR T cells secreted increasing levels of cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α
- Lack of BiTE-induced killing at basal temperatures (37℃)

Conclusion:

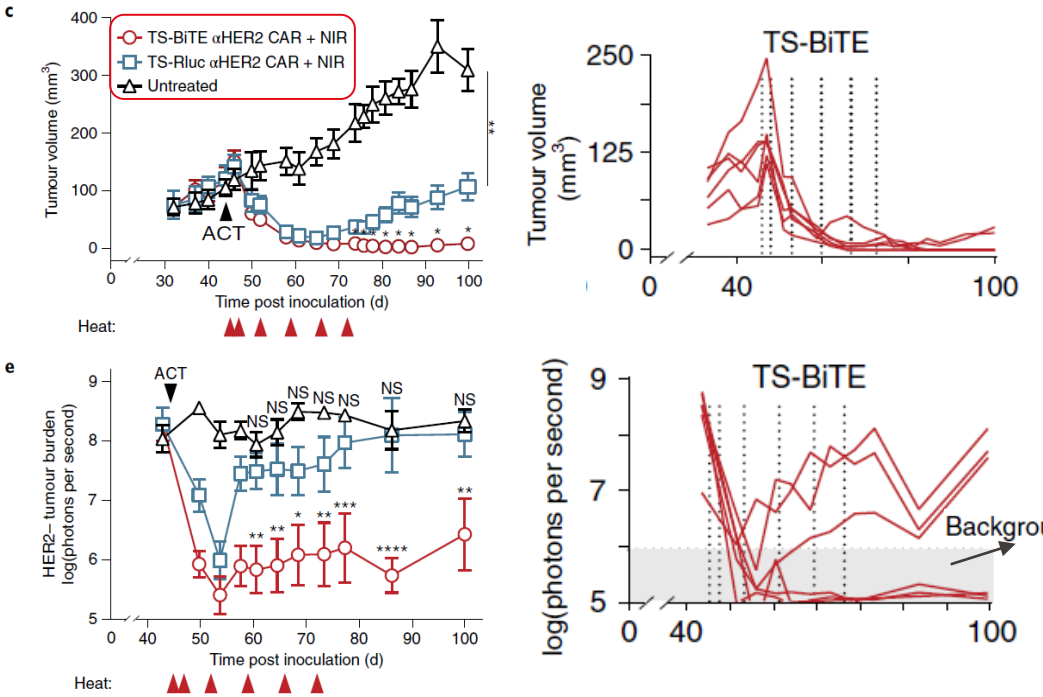
- TS-BiTE CAR T cells can be redirected to target antigen-negative tumour cells that express NKG2DL by thermal control

Next: To test mitigation of antigen escape in vivo



- Schematic of TS-BiTE and TS-Rluc αHER2 vectors

TS-BiTE αHER2 CAR T cells mitigate antigen escape



Goal: To test whether thermal control of NKG2DL BiTE could treat tumours with heterogeneous antigen expression

- A heterogenous model of breast cancer consisting of a mixture of HER2+ and HER2− tumour cells
- Inoculated NSG mice with HER2+ and HER2− cells and did ACT with NIR heating

Results:

- Significant tumour regression in mice treated with both CAR T cells
- TS-Rluc group began to relapse relative to TS-BiTE, for outgrowth of HER2− cells
- Three of six TS-BiTE mice that had impalpable tumours and luminescence within background levels for over ~45 d

Conclusion:

- Thermal control of NKG2DL BiTE has the potential to mitigate antigen escape in tumours with heterogous antigen expression

Conclusion

Photothermal targeting of engineered T cell therapies could be seen as a strategy for the improvement of responses against solid tumors

➢ **Designed synthetic thermal gene switches**

- Consists of arrays of HSEs upstream, and a core promoter
- Eliminate sensitivity to non-thermal stresses
- Response tunable depends on number of HSEs and core promoter

➢ **In mice, CAR T cells, photothermally heated, produced a transgene only within the tumors**

- Thermal control of T cell activity enhanced antitumor responses
- Thermal induction of transgenes is transient and reversible
- Thermally activated T cells remain localized
- Mitigate antigen escape

Future Work

➢ Thermal gene switches

- Explore different building parts: different number of HSEs, core promoters
- Design with lower temperature activation thresholds

➢ Heating technology

- NIR limited in penetration depth
- Focused ultrasound?

➢ Abscopal effect

- Need to study if local thermal treatment would lead to responses in distal tumours

➢ Direct comparison

- Thermally induced production of biologics v.s. systemic administration of transgenes

Critics

➢ Target only localized primary tumors

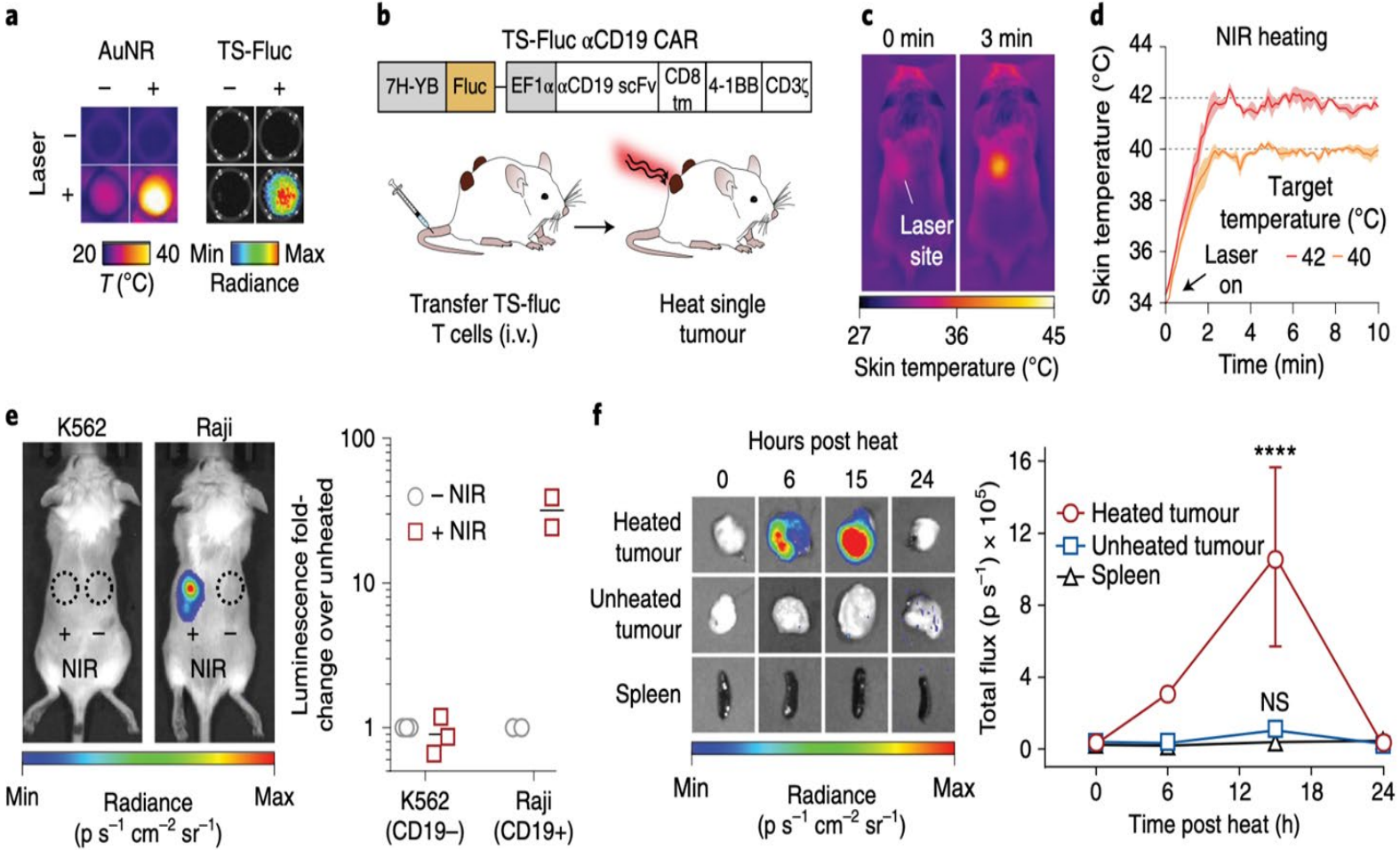
- Targeting individual metastases would preclude therapy

➢ Repeat application of heat

- Adoptive cell therapies engineered to constitutively express immunostimulatory transgenes associated with severe adverse toxicities

➢ Context specific

- Secretion rates, diffusion


a
AuNR
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b
TS-Fluc αCD19 CAR
7H-YB
Fluc
EF1α
αCD19 scFv
CD8 tm
4-1BB
CD3ζ
Transfer TS-fluc T cells (i.v.)
Heat single tumour
c
0 min
3 min
Laser site
27
36
45
Skin temperature (°C)
d
NIR heating
Skin temperature (°C)
Target temperature (°C)
Laser on
42
40
Time (min)
e
K562
Raji
NIR
Min
Radiance (p s⁻¹ cm⁻² sr⁻¹)
Max
Luminescence fold-change over unheated
– NIR
+ NIR
K562 (CD19–)
Raji (CD19+)
f
Hours post heat
Heated tumour
Unheated tumour
Spleen
Min
Radiance (p s⁻¹ cm⁻² sr⁻¹)
Max
Total flux (p s⁻¹) × 10⁵
Heated tumour
Unheated tumour
Spleen
****
NS
Time post heat (h)

Extra informations: