Screening and Characterization of AlloCAR T Targeting DLL3 for the Treatment of Small Cell Lung Cancer

Yi Zhang[1], Silvia Tacheva-Grigorova[1], Zea Melton[1], Bryan Smith[1], Tao Sai[2], Tom Van Blarcom[1], Rafael Amado[1], Barbra Sasu[1], Siler Panowski[1]

[1]Allogene Therapeutics, Inc., South San Francisco, CA, USA; [2]Formerly Pfizer Inc., South San Francisco, CA, USA

Poster #6599

Abstract

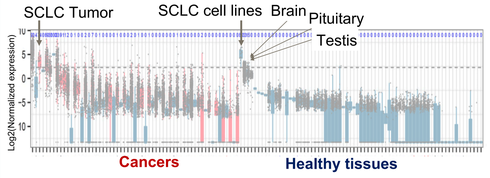
Small cell lung cancer (SCLC) is an aggressive disease with very limited treatment options. SCLC responsiveness to immuno-oncology agents suggests this indication may be amenable to a T cell based therapy. Genetically modified T cells that express chimeric antigen receptors (CARs) have shown impressive efficacy in multiple hematological malignancies. To translate this approach for SCLC treatment, we are exploring Delta-like ligand 3 (DLL3) as a therapeutic target.

A large panel of antibodies that bind to DLL3 were generated, formatted into CARs, and tested in vitro, in short-term and long-term cytotoxicity assays using target cells that express high, medium or low levels of DLL3. A subset of CAR T cells were highly active and displayed long-term killing potential. CAR T cells were engineered to contain an off-switch, by which CAR T cells are eliminated upon administration of rituximab. Multiple off-switch CAR formats were evaluated, and optimal formats determined independently. Lead DLL3 CARs in their optimal off-switch formats were tested in vivo and robust efficacy was seen in both subcutaneous and systemic SCLC models.

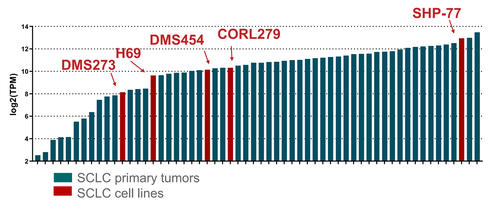
DLL3 is highly expressed in SCLC and several other types of neuroendocrine cancers, with limited normal tissue RNA expression in brain, pituitary and testis. To understand the potential for toxicity in pituitary and brain, subcutaneous or intracranial tumors expressing DLL3 were implanted in mice and human/mouse cross-reactive DLL3 CAR T cells were injected into tumor-bearing animals. T cell infiltration into intermediate and posterior pituitary was detected but no tissue damage in brain or pituitary was observed and hormone-secretion function of pituitary was not ablated. These studies decrease safety concerns associated with DLL3 CAR T.

DLL3 is expressed in SCLC with limited normal tissue expression

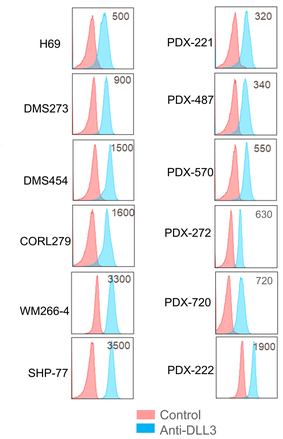
A DLL3 RNA expression is limited in normal tissues



B DLL3 RNA expression in SCLC primary tumors & cell lines



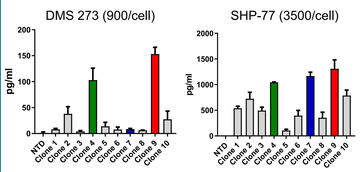
C Quantification of surface DLL3 in DLL3-positive cell lines & PDX models



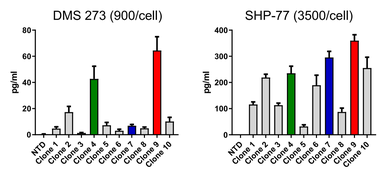
(A) RNAseq data from TCGA and GTEx shows that DLL3 is highly expressed in SCLC, but absent in almost all normal tissues except brain, pituitary and testis. (B) DLL3 RNA analysis of primary SCLC tumors and cell lines. (C) Quantification of DLL3 surface protein in DLL3-positive cell lines and PDX models.

DLL3 CAR Ts secrete cytokines and show cytotoxic activity against DLL3-positive cell lines

A DLL3 CAR Ts secrete IFNγ when co-cultured with target cells

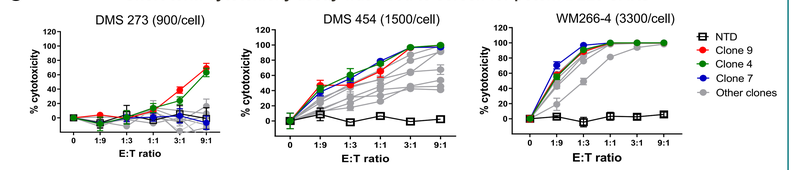


B DLL3 CAR Ts secrete IL-2 when co-cultured with target cells

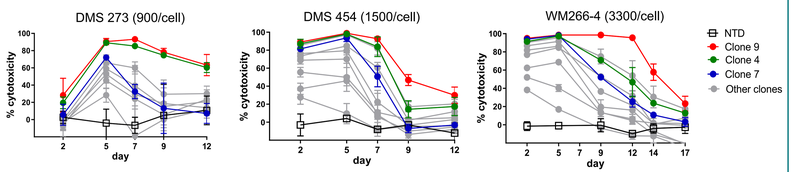


(A & B) Target cells with different DLL3 densities differentiate between DLL3 CAR Ts. Some of the clones (e.g. clone 7) secrete high levels of cytokines only with DLL3-high cell lines whereas other clones (e.g. clone 4 and 9) secrete appreciable amount of cytokines with both DLL3-low and high cell lines.

C Short-term cytotoxicity assay was used to screen for potent DLL3 CARs



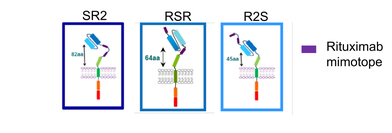
D Long-term cytotoxicity assay was used to further differentiate CARs



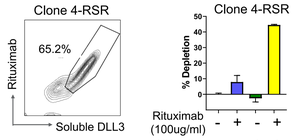
(C) DLL3 CARs were evaluated in short-term cytotoxicity assay at different E:T ratios for 72 hours. While all CARs showed activity against DLL3-med and high cell lines, clone 4 and 9 alone demonstrated cytotoxicity against the DLL3-low cell line. (D) DLL3 CARs were evaluated in a long-term stress test where CAR T cells were transferred to freshly plated target cells every 2-3 days. CARs that showed best long-term killing potential were moved into off-switch formats for further evaluation.

Optimal Rituximab-based off-switch formats were identified

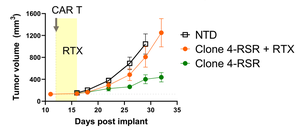
A Optimal off-switch format was chosen for lead clones based on transduction efficiency and cytotoxicity



B Rituximab-mediated CAR detection and depletion in vitro



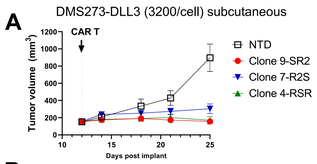
C Rituximab-mediated depletion in vivo



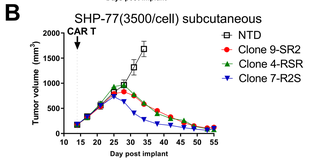
(A) DLL3 CAR Ts were engineered with three different off-switch formats and tested in the long-term cytotoxicity assay. The optimal format was determined on a per CAR basis. Three lead clones in their optimal off-switch format (clone 4-RSR, clone 7-R2S and clone 9-SR2) were moved into in vivo validation. (B) Left panel, CAR expression can be detected by flow cytometry using soluble DLL3 or rituximab. Right panel, CAR+ cells are depleted by rituximab in ADCC assay. (C) Animals treated with clone 4-RSR alone showed significant tumor inhibition, while combining rituximab with clone 4-RSR abolished the anti-tumor response. RTX, rituximab.

DLL3 CAR Ts show anti-tumor efficacy in multiple subcutaneous and systemic models

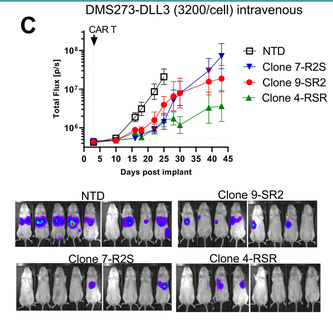
A DMS273-DLL3 (3200/cell) subcutaneous



B SHP-77(3500/cell) subcutaneous



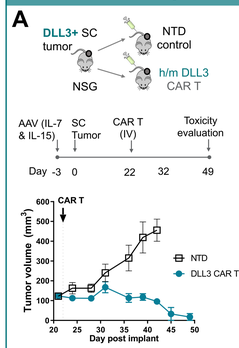
C DMS273-DLL3 (3200/cell) intravenous



(A) DLL3 CAR T cells with their optimal off-switch format are efficacious against DMS273-DLL3 subcutaneous tumor (B) Multiple DLL3 CAR T cells cleared SHP-77 subcutaneous tumor (C) Multiple DLL3 CAR T cells showed efficacy in DMS273-DLL3 systemic model that mimic metastatic disease in human patients.

Toxicity study with subcutaneous (SC) tumor showed T cell infiltration in pituitary but no tissue damage was observed

A



B Human CD3 staining (day 49)

Group	Animal	Brain	Pituitary
Control	#3	1	1
	#4	1	1
	#5	1	1
DLL3 CAR T	#1	2	3
	#2	1	3
	#3	1	3

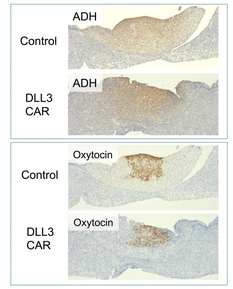
Staining score: 0 = No staining; 1 = Sparse-to-moderately low staining; 2 = Moderate-to-moderately high staining; 3 = Abundant staining

C Histopathology Analysis (day 49)

Group	Animal	Brain	Pituitary
Control	#3	No lesions	No lesions
	#4	No lesions	No lesions
	#5	No lesions	No lesions
DLL3 CAR T	#1	No lesions	Infiltrate, mixed cell (severity=3)
	#2	No lesions	Infiltrate, mononuclear cell(severity=2)
	#3	No lesions	Infiltrate, mononuclear cell(severity=2)

**Severity: 1=Minimal; 2=Mild; 3=Moderate

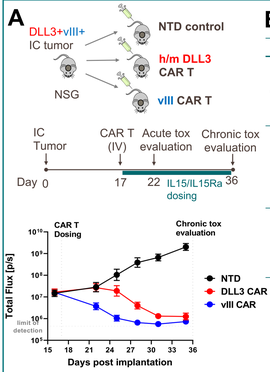
D Hormone secreting cells can be detected despite T cell infiltration in pituitaries



(A) Design and anti-tumor efficacy of in vivo study with subcutaneous tumor to understand toxicity liabilities. NTD, non-transduced T cells. (B) Human CD3 staining shows T cell infiltration in pituitary of DLL3 CAR-treated animals. (C) Histopathology analysis showed mild infiltration/inflammation in pituitary of DLL3 CAR-treated animals but no tissue damage. (D) Hormone secreting cells were not ablated despite T cell infiltration

Toxicity study with intracranial (IC) tumor showed no brain damage in DLL3 CAR-treated animals

A



B Human CD45 staining (day 36)

Group	Animal	Brain	Pituitary
Control	#3	1	1
	#4	1	1
	#7	1	1
	#8	1	1
	#9	1	1
DLL3 CAR T	#11	1	2
	#13	1/2	1/2
	#16	1	1/2
	#17	1	1/2
	#18	1	1/2
vIII CAR T	#21	1	1
	#27	1	1
	#29	1	1
	#30	1	1
	#35	1	1

hCD45 Staining score:
0 = No staining
1 = Rare/Sparse staining
2 = Sparse-to-moderately low staining
3 = Moderate-to-moderately high staining
4 = Abundant staining

C Histopathology Analysis (day 36)

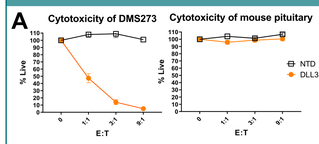
Group	Animal	Brain	Pituitary
NTD	#3	No lesions	No lesions
	#4	No lesions	No lesions
	#7	Tumor	No lesions
	#8	Tumor	No lesions
	#9	Tumor	No lesions
DLL3 CAR T	#11	Tumor	Infiltrate, mononuclear cell (severity=2)
	#13	Tumor	Infiltrate, mononuclear cell (severity=2)
	#16	Tumor	Infiltrate, mononuclear cell (severity=1)
	#17	No lesions	Infiltrate, mononuclear cell (severity=2)
	#18	No lesions	Infiltrate, mononuclear cell (severity=2)
vIII CAR T	#21	No lesions	No lesions
	#27	No lesions	No lesions
	#29	No lesions	No lesions
	#30	No lesions	No lesions
	#35	No lesions	No lesions

**Severity: 1=Minimal; 2=Mild; 3=Moderate

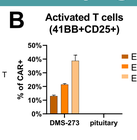
(A) Design and anti-tumor efficacy of in vivo study with intracranial tumor to understand tox liabilities. (B) Human CD45 staining shows T cell infiltration in pituitary of DLL3 CAR-treated animals. (C) Histopathology analysis showed mild infiltration/inflammation in pituitary of DLL3 CAR-treated animals. No findings for brain samples.

DLL3 CAR Ts show no cytotoxicity against mouse pituitary cells

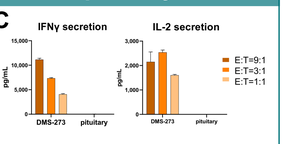
A Cytotoxicity of DMS273 / Cytotoxicity of mouse pituitary



B Activated T cells (41BB+CD25+)



C IFNγ secretion / IL-2 secretion



(A) Pituitaries from NSG mice were dissociated and co-cultured with NTD or DLL3 CAR Ts for 3 days. DLL3 CAR Ts are cytotoxic against DMS273 cells but not mouse pituitary cells. (B) Surface staining for CD25 and 41BB of the T cells co-cultured with targets, demonstrating that mouse pituitary cells do not activate DLL3 CAR Ts. (C) DLL3 CAR Ts do not secrete cytokines when co-cultured with mouse pituitary cells.

Conclusion

- DLL3 RNA is expressed in SCLC with normal tissue expression limited to brain, pituitary and testis
- CARs were screened, characterized, and ranked against targets using in vitro cytotoxicity assays
- Optimal Rituximab-based off-switch formats were determined independently for each CAR
- DLL3 CAR T cells are efficacious in multiple in vivo tumor models, including subcutaneous and systemic models
- Toxicity studies using subcutaneous and intracranial tumor models showed no tissue damage in brain or pituitary despite T cell infiltration in pituitary