# Abstract: S216

# Title: DEVELOPMENT AND EVALUATION OF A NOVEL CAR-T CELL THERAPY AGAINST CALRETICULIN MUTANT NEOPLASMS

**Abstract Type: Oral Presentation**

**Session Title: Clinical and translational in MPN**

**Background:**

Mutant calreticulin (CALR) – the driving oncogene in ~1/3 of essential thrombocythemia and myelofibrosis patients – is an attractive immunotherapy target. All oncogenic variants produce a mutant protein that aberrantly interacts with the thrombopoietin receptor (TPO-R) leading to cytokine-independent activation, and prominent display of the cancer neoantigen on the surface of TPO-R-expressing cells, including malignant stem/progenitors and megakaryocyte-lineage cells. Therapeutic antibodies against mutCALR recently entered clinical trials. Here, we report pioneering pre-clinical validation of a second-generation, 4-1BB chimeric antigen receptor T-cell (CAR-T) therapy, designed to target mutant CALR (mutCALR) driven myeloproliferative neoplasms (MPNs).

**Aims:**

To develop and evaluate a CAR-T cell therapy targeting mutCALR+ neoplasms.

**Methods:**

A binding domain was identified that specifically targets the common mutant C terminus of mutCALR, as validated using immunohistochemical staining of mutCALR+ myelofibrosis bone marrow biopsies. We engineered a suite of low and high expressing mutCALR cell lines and used these together with peripheral blood samples from patients to test the killing efficacy of the novel CAR-T in 2D liquid cultures and in 3D human bone marrow organoids, to mimic the myelofibrotic tissue environment. For in vivo evaluation, we employed NSG mice inoculated with 200,000 mutCALR+ luciferase + MARIMO cells engineered to express TPO-R to enhance cell surface neoantigen. Therapeutic efficacy was assessed through bioluminescent imaging, with tissue collection at the point of clear therapeutic success.

**Results:**

The novel CAR-T demonstrated almost complete eradication of both low- and high- mutCALR expressing MARIMO cells – a human cell line derived from mutCALR+ acute myeloid leukaemia – as well as the UT7-TPO megakaryocytic cell line engineered to express the del52 mutCALR variant. CAR-T proliferation and cytokine release was present solely in response to mutCALR-positive targets. Encouragingly, the CAR-Ts selectively depleted 25-75% of CD34+ stem/progenitor cells (HSPCs) from chronic-phase myelofibrosis patients (n=4), including both Ins5 and del52 variants, showing very high specificity and minimal toxicity to JAK2V617F+ patient cells (n=4) (Fig. A). Heterogeneity in killing efficacy was observed between patient samples, with notably lower cytotoxicity of samples from patients with accelerated/blast phase disease (n=2, Fig. A), likely reflecting both the % of mutant vs. wild-type cells and the level of expression of the mutCALR-TPO-R complex on the HSPC surface.

In NSG mice xenografted with mutCALR+ TpoR+ MARIMO cells, the CAR-Ts dramatically curtailed leukaemic burden (Fig. B) and extended survival in a different cohort where early tissue harvesting was not performed. The infiltration of CAR-T cells in the bone marrow was confirmed, together with near complete absence of tumour cells (Fig. B), affirming the ability of CAR-T to achieve remarkable ablation of mutCALR+ haematological malignancies.

**Summary/Conclusion:**

We have pioneered a first-in-class CAR-T cell therapeutic to target mutCALR-driven myeloproliferative blood

cancers. Myelofibrotic bone marrow may represent a challenging environment for cancer immunotherapy. Our ongoing work will interrogate the ability of CAR-T to ablate the mutant clone and ameliorate disease features *in vivo* and in human organoids, with a view to future clinical trial application.

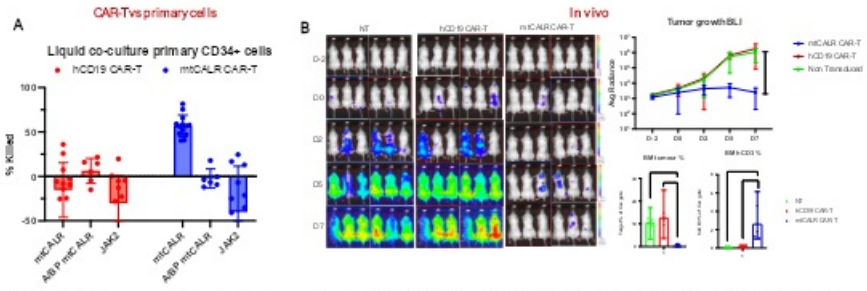


Figure. (A) CAR-Ts were generated from 4 healthy donors and co-cultured with CD34+ cells from 7 patients with chronic phase MF including mutCALR (n=4, (Ins5, n=1; del52, n=3)) and JAK2 mutations (JAK2, n=3) plus two patients with mutCALR+ myelofibrosis in accelerated or blast phase (A/BP mtCALR). Plots show % killing at 48h after co-culture of effector:target in a 1:1 ratio. (B) (Left) Bioluminescence imaging after Marimo TpoR+ cell inoculation, D0: day of CAR-T injection. (Right-top) Average radiance measured during BLI imaging. (Right-bottom) % tumour cells, and CAR-T cells in the total live bone marrow cells at day 7 post CAR-T injection. mtCALR: mutated Calreticulin, A/BP mtCALR: Accelerated phase/Blast phase mutated Calreticulin.

**Keywords:** CAR-T, Myelofibrosis, Myeloproliferative disorder