ELSEVIER

Blood 142 (2023) 4805–4806

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Adaptor Anti-P329G CAR T Cells for Modular Targeting of AML

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Introduction

Chimeric antigen receptor (CAR) T cell therapy has achieved promising results for treatment of B cell and plasma cell malignancies, but success has been limited in the treatment of acute myeloid leukemia (AML). Numerous antigens including CD33, CD123 or CSF1R have been proposed for AML therapy. However, clinical success of CAR T cell therapy in AML is impaired by antigen heterogeneity, therapy-associated toxicities, and antigen escape. Adaptor CAR T cells which allow sequential, transient or simultaneous targeting of multiple antigens have the potential to overcome these limitations. These adaptor CAR T cells were combined with tumor antigen-directed CAR-adaptor molecules for indirect tumor binding. We developed a modular P329G-directed CAR targeting the P329G mutation in the Fc part of tumor-targeting human IgG1 antibodies containing P329G L234A/L235A (PGLALA) mutations. These PGLALA mutations render the Fc part inactive for binding to Fc gamma receptors (FcgRs) and to the complement system, and up to now, more than 20 such Fc-silenced therapeutic antibodies including an approved T cell recruiting bispecific antibody have been clinically validated. Compared to other modular CAR T cell platforms this approach does not rely on haptens or artificial tags fused to the targeting antibody and may ease development and testing of the platform.

Methods

A scFv-based second generation CAR vector system was used in primary human T cells. P329G-targeting CAR T cells were combined with PGLALA-Fc-mutated antibodies targeting CD33, CD123 or CSF1R. Classical, directly binding anti-CD33 and anti-CSF1R CAR T cells have been used as positive controls, while anti-CD19 CAR T cells and untransduced T cells have been used as negative controls. Different AML (MOLM-13, OCI-AML-3, PL-21, Mv4-11, THP-1 and U-937) and an ALL cell line (NALM-6) were used as target cell lines expressing the target antigens in different intensity. Effector function of CAR T cells was also tested against primary human AML blasts derived from patients. Luciferase-based killing assays, ELISA-based and flow-based analysis were used to evaluate CAR effector functions.

Results

Unlike CD16-CAR T cells, which bind human IgG in a non-selective manner, anti-P329G CAR T cells revealed specific effector functions only when combined with antibodies carrying PGLALA-Fc-mutations. Anti-P329G CAR T cells alone revealed no activation. Anti-P329G CAR T cells could be specifically activated by recombinant protein when combined with antibodies targeting CD33, CD123 and CSF1R leading to upregulation of T cell activation markers. Anti-P329G CAR T cells combined with PGLALA-mutated antibodies targeting CD33, CD123 und CSF1R achieved efficient *in vitro* cytotoxicity against tested AML cell lines and primary human AML blasts, while antigen-negative ALL cell line NALM-6 was not killed. Remarkably, effector functions of anti-P329G CAR T cells were similar to classical CAR T cells particularly when targeting CD33. This led to specific cytokine production when anti-P329G CAR T cells were co-cultured with respective antibodies and tumor cells. P329G-CAR T cells activated by recombinant CD33, CD123 or CSF1R showed no cytokine production in the presence of an antibody which

was not recognizing the respective antigen. However, by switching the antibody after 24 h of stimulation towards the tumor antigen-targeting antibody, CAR T cell activation could be again achieved by the recombinant protein. Depleting the tumor antigen-targeting antibody after 24 h of stimulation decreased the cytokine production after 48 h and 72 h despite ongoing presence of the recombinant protein. This confirms the modularity and reversibility of this adaptor CAR T cell platform.

Conclusions

Taken together, anti-P329G CAR T cells combined with Fc-silenced tumor antigen-targeting IgG1 antibodies carrying the clinically validated PGLALA-mutations in the Fc part achieved profound effector functions against various human AML cell lines and primary AML blasts. The modular platform has the potential to overcome recent limitations of CAR T cell therapy in AML. These results support the rational for clinical translation of this novel and modular CAR T cell platform.

Disclosures Stock: *Novartis:* Other: Award for young scientists. **Gottschlich:** *LMU University Hospital, LMU Munich:* Patents & Royalties; *Tabby Therapeutics:* Research Funding. **Surowka:** *Roche:* Current Employment, Patents & Royalties. **Bruenker:** *Roche:* Current Employment, Other: Stock ownership, Patents & Royalties. **Darowski:** *Roche:* Current Employment. **Endres:** *TCR2 Inc.:* Other: License fee, Research Funding; *Carina Biotech:* Other: License fee; *LMU University Hospital, LMU Munich:* Patents & Royalties; *Tabby Therapeutics:* Research Funding; *Plectonic GmbH:* Research Funding; *Arcus Bioscience:* Research Funding. **Subklewe:** *Novartis:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Ichnos Sciences:* Consultancy, Honoraria; *Roche:* Consultancy, Honoraria, Other: Travel Support, Research Funding, Speakers Bureau; *Pfizer:* Consultancy, Honoraria, Other: Travel Support, Speakers Bureau; *AstraZeneca:* Speakers Bureau; *BMS/Celgene:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Gilead/Kite:* Consultancy, Honoraria, Other: Travel Support, Research Funding, Speakers Bureau; *Janssen:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Miltenyi Biotec:* Consultancy, Honoraria, Research Funding; *Takeda:* Consultancy, Honoraria, Research Funding; *Seagen:* Research Funding; *Amgen:* Consultancy, Honoraria, Research Funding; *AvenCell:* Consultancy, Honoraria; *Incyte Biosciences:* Consultancy, Honoraria; *Molecular Partners:* Consultancy, Honoraria, Research Funding; *GSK:* Speakers Bureau; *LAWG:* Speakers Bureau; *Springer Healthcare:* Speakers Bureau; *AbbVie:* Consultancy, Honoraria; *Autolus:* Consultancy, Honoraria; *advesya (CanCell Therapeutics):* Consultancy, Honoraria; *Genmab US:* Consultancy, Honoraria; *Interius BioTherapeutics:* Consultancy, Honoraria; *Nektar Therapeutics:* Consultancy, Honoraria; *Orbital Therapeutics:* Consultancy, Honoraria; *Sanofi:* Consultancy, Honoraria; *Scare:* Consultancy, Honoraria. **Klein:** *Roche/Genentech:* Current Employment, Current equity holder in publicly-traded company, Other: Stock ownership, Patents & Royalties. **Kobold:** *Plectonic:* Research Funding; *Miltenyi Biotech:* Honoraria; *Tabby Therapeutics:* Research Funding; *Arcus Biosciences:* Research Funding; *Carina Biotech:* Other: IP License; *TCR2 Inc:* Honoraria, Other: IP License, Research Funding; *Novartis:* Honoraria; *GSK:* Honoraria; *BMS:* Honoraria.

https://doi.org/10.1182/blood-2023-182864