ELSEVIER

Blood 142 (2023) 6805–6806

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Development of Chimeric Antigen Receptor (CAR) T Cells Targeting MET in Lymphomas and Solid Tumors

Pohan Chen, MDPhD[1], *Rianna Raghunandan*[2], *Markus Müschen, MD*[3], *Samuel G. Katz, MDPhD*[1]

[1] Department of Pathology, Yale School of Medicine, New Haven, CT
[2] Yale College, New Haven, CT
[3] Center of Molecular and Cellular Oncology, Yale Cancer Center, Yale School of Medicine, New Haven, CT

Currently, multiple versions of CAR T cells targeting the cell surface protein CD19 are approved by the FDA to treat refractory B-cell malignancies. However, durable remissions range from 60% in B-cell leukemias to 40% in B-cell lymphomas. Major reasons for resistance to CD19-CAR treatment relate to heterogeneous or loss of CD19 expression. In addition, CD19-CAR leads to B-cell depletion which increases the risk for infection. These limiting factors necessitate research into alternative targets for CAR therapy against B-cell lymphomas.

While HGF/MET signaling has been extensively studied in solid tumors, studies have also shown important roles of HGF/MET in hematologic malignancies. Approximately 30-70% of diffuse large B-cell lymphomas (DLBCL) express MET with moderate to strong intensity; as do other lymphomas such as classic Hodgkin lymphoma, Burkitt lymphoma, primary effusion lymphoma, and multiple myeloma. We hypothesize that these MET-positive B-cell lymphomas can potentially be targeted with chimeric antigen receptor (CAR) T-cells against MET receptor.

We first looked at the DepMap database and found that a significant subset of aggressive and large B-cell lymphoma cell lines express MET such as Raji, Karpas-422, OCI-LY3, and OCI-LY132. Of note, some of them, like OCI-LY3 and OCI-LY132, lack detectable CD19. Flow cytometry using anti-MET antibody confirmed MET expression in these cell lines. Further analysis of RNAseq data from 500 CD19-CAR T-naïve primary patient samples (GSE125966) revealed MET expression in 62% (294/474) of CD19-positive and 61% (15/26) of CD19-low to negative DLBCL **.**

We then constructed a second-generation anti-MET-CAR by fusing the scFv fragment of an anti-MET monoclonal antibody with the human CD28 hinge/transmembrane/cytoplasmic domain and CD3z cytoplasmic domain. Stable MET-CAR Jurkat T cells were incubated with various target lymphoma cells for 16-24 hours, and the percentage of Jurkat T cells expressing the activation marker CD69 was determined by flow cytometry. MET-CAR Jurkat showed increased CD69 expression in MET-positive lymphoma cell lines such as Karpas-422 and OCI-LY3. Karpas-422 demonstrated dose dependent activation of MET-CAR Jurkat cells, with higher CD69 expression in response to increasing amounts of MET antigen. OCI-LY3, a lymphoma cell line negative for CD19 but positive for MET, led to only basal level of CD69 expression in CD19-CAR Jurkat but elevated CD69 level in MET-CAR Jurkat.

After showing MET-CAR Jurkat T cell activation by lymphoma cell lines, we tested cytotoxicity using primary human CD3+ T-cells against A549, a lung cancer cell line with known high level of MET expression, as a positive control for MET-CAR mediated cytotoxicity. Activated primary human T cells were electroporated with MET-CAR mRNA. Twelve hours post electroporation, the reprogrammed T cells were mixed with A549 cells at a 5:1 effector: target ratio (E:T). CellCyte proliferation analysis showed reduced A549 cell proliferation in the presence of MET-CAR T cells, in comparison to mock electroporated CD3+ T cells. We further demonstrated that the same mRNA MET-CAR T-cell was able to mediate substantial cytotoxicity against DLBCL cell lines such as Karpas-422 and OCI-LY3 (**Figure 1**). At varying E:T ratio, MET-CAR T cell exhibited significant cytotoxicity against Karpas-422. Also, consistent with the CD69 expression data, while CD19-CAR T cell showed only low level of cytotoxicity against the CD19-/MET+ OCI-LY3 cell line, MET-CAR T cell was able to mediate substantial tumor cell lysis. *In vivo* testing against a xenograft model is currently underway.

In summary, the preliminary data demonstrate that MET-CAR is capable of mediating cytotoxicity against MET-positive DLBCL. Some cell lines such as OCI-LY3 are negative for CD19 but positive for MET expression, and MET-CAR is capable of mediating cell lysis while CD19-CAR is ineffective. These results suggest that some large B-cell lymphomas negative for CD19, either intrinsic or secondary to loss of CD19 as a result of resistance mechanism, may show MET expression and will likely benefit from MET-targeted immunotherapy.

Figure caption:

Figure 1. Cytotoxicity of MET-CAR T cell against DLBCL cell lines. (A) MET-CAR against Karpas-422 at various E:T ratio. **(B)** Cytotoxicity of MET-CAR against OCI-LY3 which is positive for MET but negative for CD19.E:T ratio is 10:1.

Disclosures No relevant conflicts of interest to declare.

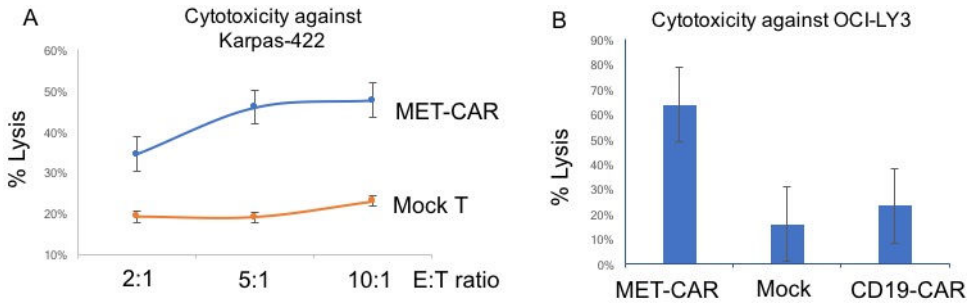

Figure 1

https://doi.org/10.1182/blood-2023-186161