Activated conformation of integrin-β7 is a new myeloma-specific target for immunotherapy and CAR T-cell specificity

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The ultimate goal of cancer research is the identification of a unique target on cancer cells that can be utilized for directed treatment, leaving normal cell populations unharmed. Due to the clonal heterogeneity in multiple myeloma (MM), this isn't an easy task, as specific targets can change as the disease progresses and there are often differences between individual patients. Searching for such targets has been made easier by transcriptome or proteasome analyses, but these will often miss proteins with post-translational modifications or conformation-dependent active sites.

In a study carried out by Naoki Hosen, from Osaka University Graduate School of Medicine, Osaka, Japan, and a large team of researchers, cancer-specific monoclonal antibodies (mAbs) were identified, and they worked backward to identify the relevant antigens. Their findings were published in *Nature Medicine* in November 2017.

**Key Highlights:**

- More than 10,000 hybridoma clones were established that bound to MM cell lines
- In a first screen, antibodies that did not bind to peripheral blood mononuclear cells (PBMCs) from unaffected donors were eliminated, leaving approximately 500 clones
- A second screening of these 500 clones identified monoclonal antibodies (mAbs) that bound to MM cells, but not CD45+ leukocytes in bone marrow (BM) of MM patients, and clone MMG49 was identified
- Antigen specificity of MMG49 was delineated using a cDNA library expressed in MM.1S cells and subsequent sequencing of the reactive population, identifying integrin-β7 as the relevant epitope
- Selective binding to integrin-β7 was confirmed by immunoprecipitation, ELISA, and flow cytometry
- Cellular staining experiments suggested that MMG49 could only bind to the active conformation of integrin-β7, and this was confirmed by epitope mapping
- Integrin-β7 was found to be over-expressed on MM cells, and in the constitutively active conformation with high reactivity to MMG49; conversely, BM cells from healthy donors had limited reactivity to MMG49 (only CD38+CD138+ plasma cells were reactive)
- GGM49 did not elicit antibody-mediated toxicity due to a low density of the epitope on MM cells, but the respective epitope was pursued as a candidate for CAR T-cell development
- MMG49 CAR was generated by cloning the single chain variable fragment (scFv) from MMG49 into a CAR-T construct containing the CD28 and CD3zeta signaling domains
- Subsequent transduction of T cells led to the efficient production of MMG49 CAR T-cells which proliferated exponentially, produced cytokines, and exhibited cytotoxicity towards BM cells from MM patients
Infusion of MMG49 CAR T-cells into a xenograft mouse model led to significantly decreased tumor burden and prolonged survival using a luciferase-expressing MM.1S model ($P < 0.0001$) as well as an RPMI8226 model of subcutaneous injection ($P < 0.0001$) (both using NOG mice).

No unexpected side-effects were observed.

This pre-clinical study outlines highly promising data showing that the active conformation of integrin-β7 can be used as an immunotherapeutic target to generate MM-specific CAR T-cells. The researchers used a novel methodology to search for epitopes that could be missed by other screening methods, opening up the possibility of further as yet undiscovered epitopes that are cancer-specific only in their active state. The murine experiments with MMG49 CAR T-cells pave the way for further development of this CAR-T construct, such as fully humanizing the single chain variable fragment and entry of the CAR-T construct into clinical trials.

References