TARGETING MYELOID CELLS AND MODULATION OF THEIR FUNCTION BY FULLY SYNTHETIC ANTIBODY MIMETICS

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In certain circumstances, monocytes, especially macrophages, contribute to the onset of autoimmune diseases, are involved in various inflammatory disorders, and also have significant impact on the development and progression of tumors, metastasis as well as drive tumor microenvironment towards suppression of immune responses. As a result, numerous therapeutic strategies are currently being investigated to target and modulate the activity along with the specific function of these myeloid cells. One of the promising areas of investigation within therapeutic development is a specific targeting of the high affinity IgG FcγRI receptor, CD64, primarily expressed on monocytes and macrophages.

Recently, we have introduced the development of fully synthetic antibody mimetics called iBodies. These conjugates based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer have gained captivated consideration in the fields of polymer-drug delivery and biomedicine. iBodies are non-immunogenic, stable, and highly modular copolymers able to passively target tumor tissues through the enhanced permeability and retention effect. In addition, iBodies excel in their versatility, low production cost, easy preparation, and fine-tuning of their pharmacokinetics and pharmacodynamics.

The anti-CD64 iBodies proved a significant improvement in binding potency to CD64, compared to the CD64 ligand alone. Using flow cytometry and confocal microscopy, we detected a specific binding of anti-CD64 iBodies to human monocytes and monocytes-derived macrophages even with subnanomolar effectiveness. The anti-CD64 cytotoxic iBodies, simultaneously decorated with a specific ligand targeting CD64 and a cytotoxic moiety connected to the HPMA copolymer by a cleavable linker, showed selective depletion of $CD64^{-positive}$ cells compared to the control cytotoxic iBodies without the CD64 ligand and $CD64^{-negative}$ cells. Furthermore, iBodies proved limited cytotoxicity and no off-target specificity within

a whole fraction of peripheral blood mononuclear cells *in vitro*. In monocyte-derived macrophages, the anti-CD64 cytotoxic iBodies modulate their immune function by causing cell death pointing towards apoptosis, alter the anti-inflammatory phenotype and interfere with binary M1/M2 macrophage polarization.

In conclusion, we have developed anti-CD64 cytotoxic iBodies that are able to specifically eliminate monocytes and macrophages and modulate their phenotype. iBodies may provide novel therapeutical strategies and opportunities for drug development and delivery in cancer immunotherapy.