Abstract #61 | Poster

In vivo production of bispecific antibodies against hematological malignancies

Y. Yang[1], M. Freihammer[1], R. Flümann[1], A. Lütz[1], J. Petri[1], M. Michalik[1], S. Borchmann[1, 2]

[1] *the University of Cologne, Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, Cologne, Germany*
[2] *German Hodgkin Study Group, Cologne, Germany*

Background:
Bispecific T cell engagers (BiTEs) are effective but limited by an extremely short serum half-life (~2 hours), requiring continuous infusion. While protein engineering can extend stability, it often increases manufacturing complexity. We developed an mRNA-based platform for the in vivo expression of bispecific antibodies (bsAbs) to provide sustained expression of bispecific antibodies.
Objectives:
1. Design DNA templates for in vitro transcription of stabilized mRNA constructs.
2. Verify protein production by transfecting mRNA into various cell lines.
3. Evaluate target-dependent cytotoxicity mediated by in vitro–transcribed mRNA encoding bispecific antibodies.
4. Monitor plasma levels of functional proteins and assess tumor regression in xenograft mouse models treated with bsAb-encoding mRNA.
Methods:
mRNA was engineered with a 5′ cap, optimized UTRs, and a poly(A) tail, then transfected into cells using lipid nanoparticles (LNPs). Secreted bsAbs were analyzed via Western blot and flow cytometry. Functional potency was assessed in human PBMC–tumor co-cultures by toxicity assay, T cell activation (CD25/CD69), and cytokine release (IFN-γ/IL-2). Finally, NSG xenograft mice were treated with mRNA or purified protein to compare tumor regression and pharmacokinetics.
Results:
mRNA transfection led to robust secretion of functional bsAbs with high binding specificity. In co-cultures, mRNA-derived bsAbs induced potent, antigen-specific tumor lysis and significant T cell activation. Currently, in vivo studies demonstrated that DNA-based delivery provided significant tumor growth inhibition in NSG mice. In contrast, purified bsAbs failed to show any inhibitory effect. Experiments specifically evaluating the in vivo efficacy of the mRNA delivery system in the same NSG model are currently ongoing.
Conclusions:
Our results show that mRNA is efficiently translated into functional bispecific antibodies in vitro. Early in vivo data confirmed efficacy, and ongoing studies are further evaluating the in vivo efficacy and safety of this mRNA-based approach in murine tumor models.
Keywords: mRNA Therapeutics, Immunotherapy