blocking counterregulation of unfolded protein response by targeted protein synthesis inhibition produces highly synergistic cell death in several cancer entities

Background Because tumor cells have high proliferation rates the demand for energy on the one hand and proteins on the other hand is high. In line, protein folding machinery of the ER is heavily used. 2-Deoxyglucose (2-DG) not only blocks energy synthesis by inhibiting glycolysis but also blocks synthesis of mannosyl leading to impaired N-linked glycosylation, accumulation of misfolded proteins, and increased unfolded protein response (UPR). However, due to compensatory events, UPR-induced apoptosis is hampered. Therefore, we combined 2-DG with targeted protein synthesis inhibition by immunotoxins, consisting of an antibody and pseudomonas exotoxin, to enhance UPR mediated cell death.

Materials and Methods Established cell lines and patient-derived B-ALL samples were treated in vitro with various protein synthesis inhibitors and UPR-inducers. Drug synergy was determined mathematically as fold-increase over additivity.

Biochemical studies were performed using western blots. In vivo enhancement was tested using systemic xenograft models.

Results The combination of Moxetumomab and 2-DG achieved a two to nine-fold synergy in vitro. Synergy was abrogated by the addition of Mannose suggesting UPR as cause of synergistic cell death. Similarly, Moxetumomab enhanced UPR-inducers Bortezomib and tunicamycin and protein synthesis inhibition by cycloheximide and puromycin enhanced 2-DG suggesting a conserved mechanism. Using HB21, an immunotoxin targeting human transferrin-receptor, breast cancer, hepatocellular carcinoma, and glioblastoma were sensitized to 2-DG induced cell death. Biochemically, 2-DG increased XBP-1-cleavage, expression of pro-apoptotic CHOP and of anti-apoptotic BIP. Moxetumomab, however, blocked the upregulation of BIP while maintaining CHOP correlating with synergistic increase in PARP-cleavage and apoptosis. In two systemic mouse models, bone marrow (BM) lymphoma infiltration was not reduced by 2-DG or tunicamycin alone but was reduced after treatment with Moxetumomab alone by 5-fold in the JeKo-1 and by 16-fold in the Ramos model respectively. The combination of Moxetumomab and 2-DG achieved a three-fold synergy in the JeKo-1 model and achieved MRD-negative BM status in the Ramos model. Against patient-derived B-ALL of the Burkitt’s type, 2-DG and Moxetumomab were up to 5-fold more active in vitro and up to 7-fold more active in mouse xenografts in vivo.

Conclusions Cell death after persisting unfolded protein response is synergistically enhanced by tumor-cell specific inhibition of protein synthesis against four distinct tumor entities at physiologically achievable concentrations. Our approach of immunotoxin-induced targeted protein synthesis inhibition opens a novel, so far undescribed therapeutic window which may warrant clinical evaluation.