HIGHLY EFFICIENT SMALL MOLECULE INHIBITORS OF LMP1-TRAF2 INTERACTION BLOCK LMP1 SIGNALING AND INTERFERE WITH LCL AND PTLD SURVIVAL

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Introduction: The latent membrane protein 1 (LMP1) is involved in the development of Epstein-Barr virus (EBV)-associated post-transplant (PTLD) and Hodgkin's lymphoma as well as nasopharyngeal carcinoma. LMP1 activates cellular signaling by recruiting members of the cellular TNF receptor-associated factor (TRAF) protein family to its C-terminal activation regions (CTAR) 1 and 2. The interaction of TRAF2 with CTAR1 is critical for signal transduction by LMP1 and the survival of EBV-transformed B cells.

Objectives: We aim at developing specific small molecule inhibitors that disrupt the LMP1–TRAF2 protein-protein interaction (PPI) and thereby block LMP1 signaling to induce cell death of EBV-transformed tumor cells.

Methods: We have established industry-standard AlphaScreen-based PPI high throughput screening technology for the LMP1–TRAF2 interaction using recombinant GST-LMP1 and His-TRAF2 proteins. Novel derivatives of the primary screening hit EN254 were synthesized and tested in activity and ADME-pharmacokinetics assays to optimize the EN254 inhibitor class in a hit-to-lead development process towards a preclinical lead compound. LMP1–TRAF2 inhibitors were tested for their effects on the survival lymphoblastoid and PTLD cells in MTT assays. LCLs expressing the inducible NGFR-LMP1 fusion instead of wildtype LMP1 were used to demonstrate the specific inhibitory effects of EN254 derivatives on LMP1-induced NF-kappaB and JNK signaling as well as the LMP1–TRAF2 interaction.

Results: 350,000 synthetic small molecules were screened for inhibitors of the LMP1–TRAF2 PPI. Starting from the primary hit EN254, more than two hundred EN254 derivatives have been tested in iterative rounds of activity measurements and novel synthesis to establish a structure-activity relationship and to improve the activity of the compounds versus LMP1–TRAF2. Novel chemical matter has been developed. The current lead compound SBL316 blocks the LMP1–TRAF2 PPI at an IC50 concentration in the nanomolar range, whereas it shows no effect on LMP1–TRAF6. SBL316 inhibits the lipid raft recruitment of TRAF2 mediated by LMP1. Furthermore, LMP1–TRAF2 PPI inhibitors efficiently interfere with NF-kappaB and JNK activation by NGFR-LMP1 as well as the survival of LCLs and patient-derived PTLD cells at nanomolar concentrations. SBL316 derivatives show low toxicity and promising results in ADME studies. Pharmacokinetics studies are underway to prepare proof-of-concept studies of the inhibitors in a preclinical xenograft mouse model of PTLD.

Conclusion: We have established the first potent small molecule LMP1–TRAF2 inhibitors, which efficiently interfere with LMP1 function and will be further developed towards an anti-EBV drug.