MKNs by eFT508 can modulate anti-tumor immunity.

Mitogen-Activated Protein (MAP) Kinase signaling cascades play a vital role in T-cell activation upon antigen recognition. MKN1 and MKN2 are important downstream effector kinases in the MAPK pathway that largely function in regulating the expression of important signaling molecules including cytokines and immune checkpoint receptors. MKNs are primarily thought to regulate the expression of select mRNAs, predominantly translation initiation factor eIF4E and the RNA binding proteins hnRNPA1 and PSF. eFT508 is a potent and highly selective inhibitor of MNK1 and MNK2 that has been shown to inhibit various important signaling molecules including cytokines and immune checkpoint receptors. In order to identify key T-cell components that are regulated by MNK inhibition and may mediate the effects of eFT508 treatment, we performed an unbiased phosphoproteomic analysis of T-cells during the early stages of T-cell receptor mediated stimulation with and without eFT508 treatment. Primary human T-cells were pre-treated with eFT508 for two hours prior to stimulation with anti-CD3/anti-CD28 for an additional 30 minutes. Protein samples were then collected for analysis. Furthermore, there was enrichment for specific sequences surrounding the phosphorylated sites in the eFT508 sensitive peptides, highlighting a potential mechanism mediating MNK target recognition. Confirmation of MNK-mediated phosphorylation of novel substrates is being conducted in vitro by biochemical analysis of direct phosphorylation of potential substrates by MKN1 or MKN2 and in cell lines treated with eFT508 to western blot analysis using phospho-specific antibodies. These findings have significantly expanded our understanding of cell signaling through MNK1 and MNK2 and will help to guide therapeutic development through which inhibition of MNKs by eFT508 can modulate anti-tumor immunity.

Conclusions

- eFT508 inhibition of MKN1 and MKN2 selectively controls the translation of key regulators of the anti-tumor immune response.
- eFT508-sensitive phosphoproteins are enriched among functional networks including mTOR signaling and mRNA translation, mRNA stability, and mRNA splicing.
- eFT508 modulates the phosphorylation of additional translation initiation factors that cooperate with eIF4E to selectively regulate translational output.
- eFT508 is currently being evaluated in phase I/II clinical trials in patients with solid tumors (NCT02605083) and lymphoma (NCT03237675) as well as a single agent or combination with axelimab in MSS colorectal cancer (NCT03258398).

Figure 1. eFT508 selectively regulates the expression of key immunomodulatory factors in activated T cells. A) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. B) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. C) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. D) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. E) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. F) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. G) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. H) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. I) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. J) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. K) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. L) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. M) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. N) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. O) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. P) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. Q) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. R) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. S) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. T) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. U) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. V) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. W) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. X) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. Y) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours. Z) Primary human T-cells were stimulated with recombinant cytokines in the presence or absence of eFT508 for 12 hours.