Identifying ERK/MAPK mediated mRNA translation elongation as a therapeutic strategy in CLL

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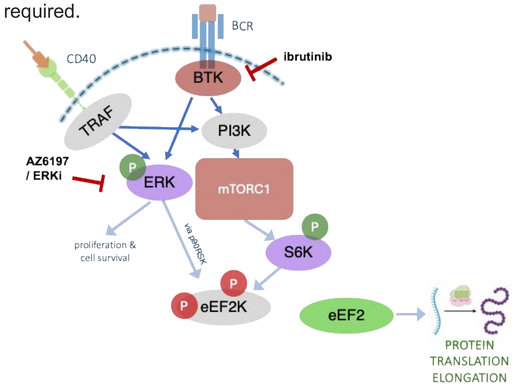
BACKGROUND

Therapies such as ibrutinib (IBR) that inhibit BTK, a key component of the BCR pathway, have revolutionised the clinical treatment of CLL, improving survival rates of poor prognostic patients. However, these treatments are not universally suitable, with the development of resistance mutations presenting as a significant problem with patients ultimately relapsing, therefore new treatment strategies are required.

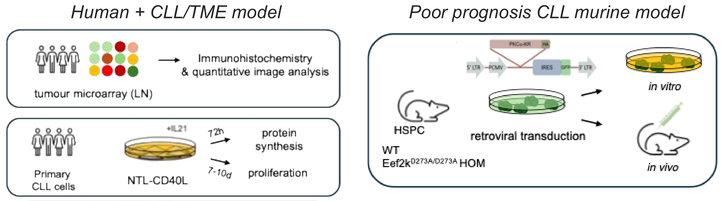


Our previous studies[1] highlighted the importance of mRNA translation elongation as a driver of disease progression, identifying inactivation of eEF2 activity as a novel therapeutic target for blocking CLL progression. eEF2 activity can be mediated by a number of post-translational modifications, most notably through phosphorylation by eEF2K[2]. eEF2K inhibition, through phosphorylation at residue Ser366 by p90RSK and p70S6 kinase or by SAPK4/p38delta at Ser359, can promote protein synthesis. This occurs in part through the reduction in eEF2^{T56} phosphorylation, thereby increasing eEF2 activity and promoting translation elongation. Here we demonstrate that targeting both ERK and BTK signalling simultaneously, to therapeutically block protein synthesis via eEF2 signalling, is an attractive clinical strategy.

HYPOTHESIS

Dual targeting of BTK and ERK-MAPK signalling to block protein synthesis is an attractive clinical strategy.

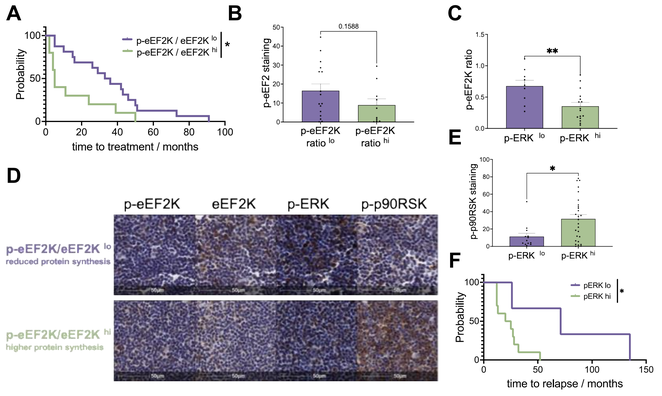
MATERIALS & METHODS



*Statistics: Data expressed as the mean ± SEM. Statistics calculated by t-test, one-way or two-way ANOVA, where * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.*

RESULTS

High eEF2K activity correlates with faster time to treatment in CLL & active ERK1/2

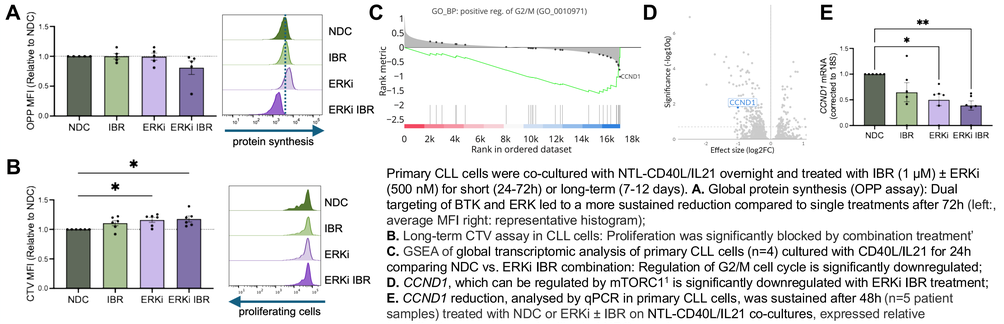


IHC was performed on LN biopsies from CLL patients (n=43 - minimum of 3 technical replicates).
A. TMA staining showed patients with above average (n=10) staining for p-eEF2K^{S366}/eEF2K have a significantly faster time to death compared to below average (n=16);
B. High p-eEF2K^{S366}/eEF2K correlates with hi peEF2^{T56} (n=10) vs. lo peEF2^{T56} (n=16); and lo pERK$^{T202/204}$ (**C**);
D. Representative images for patients in p-eEF2K^{S366}/eEF2K hi or lo cohorts with corresponding images for pERK$^{T202/Y204}$ and p-p90RSK^{S380};
E. Hi pERK$^{T202/204}$ correlates with hi p-p90RSK^{S380} (n=27) vs. lo (n=13);
F. Patients with hi pERK$^{T202/204}$ (n=10) relapsed significantly faster than those with lo pERK (n=3) (above and below average staining respectively).

REFERENCES :

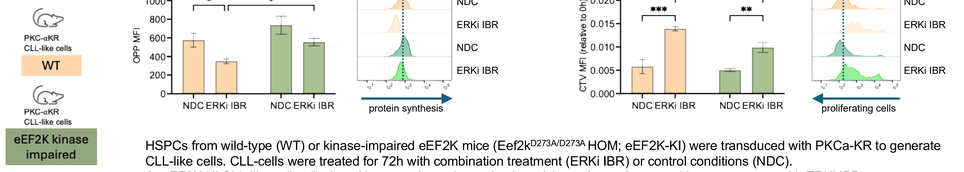
[1] Malik N, Hay J, Almuhanna HNB, et al. mTORC1-selective activation of translation elongation promotes disease progression in chronic lymphocytic leukemia. *Leukemia*. 2023;37(12):2414-2425. doi:10.1038/s41375-023-02043-3
[2] Wang X, Regufe da Mota S, Liu R, et al. Eukaryotic elongation factor 2 kinase activity is controlled by multiple inputs from oncogenic signaling. *Mol Cell Biol*. 2014;34(22):4088-4103. doi:10.1128/MCB.01035-14

ERKi can reduce protein synthesis and impede mTORC1-mediated cell cycle progression



Primary CLL cells were co-cultured with NTL-CD40L/IL21 overnight and treated with IBR (1 μM) ± ERKi (500 nM) for short (24-72h) or long-term (7-12 days). **A.** Global protein synthesis (OPP assay): Dual targeting of BTK and ERK led to a more sustained reduction compared to single treatments after 72h (left:, average MFI right: representative histogram);
B. Long-term CTV assay in CLL cells: Proliferation was significantly blocked by combination treatment'
C. GSEA of global transcriptomic analysis of primary CLL cells (n=4) cultured with CD40L/IL21 for 24h comparing NDC vs. ERKi IBR combination: Regulation of G2/M cell cycle is significantly downregulated;
D. *CCND1*, which can be regulated by mTORC1[1] is significantly downregulated with ERKi IBR treatment;
E. *CCND1* reduction, analysed by qPCR in primary CLL cells, was sustained after 48h (n=5 patient samples) treated with NDC or ERKi ± IBR on NTL-CD40L/IL21 co-cultures, expressed relative to *18S* reference gene and calibrated NDC samples.

Kinase impaired eEF2K increases protein synthesis and reduces sensitivity to BTKi/ERKi



HSPCs from wild-type (WT) or kinase-impaired eEF2K mice (Eef2k$^{D273A/D273A}$ HOM; eEF2K-KI) were transduced with PKCa-KR to generate CLL-like cells. CLL-cells were treated for 72h with combination treatment (ERKi IBR) or control conditions (NDC).
A. eEF2K-KI CLL-like cells, displayed increased protein synthesis activity and were less sensitive to treatment with ERKi IBR;
B. Proliferation of CLL-like was significantly blocked by combination treatment after 72h in WT cells, as determined with the CTV assay. However, eEF2K-KI CLL-like cells were significantly less sensitive to ERKi IBR treatment compared to WT CLL-like cells.

CONCLUSIONS

We show evidence that targeting eEF2 function/activity represents a promising therapeutic target in CLL:

- Less active eEF2K (p-eEF2K^{S366}/eEF2K)^{hi} is associated with a **more aggressive disease** in patient LN biopsies and also **more active ERK signalling**
- Treating CLL cells in *ex vivo* CLL/TME modelling, with combination ERKi/IBR **inhibits protein synthesis** and **proliferation.**
- ERKi/IBR combination induces a **G1 cell cycle arrest** and reduces **mTORC1-mediated translation of *CCND1*.**
- eEF2K-KI CLL-like mouse cells exhibit **increased protein synthesis** and reduced sensitivity to BTKi/ERKi.