3195

DYRK1B inhibitors prevent pharmacologic quiescence and sensitize lung cancers to EGFR TK inhibitors

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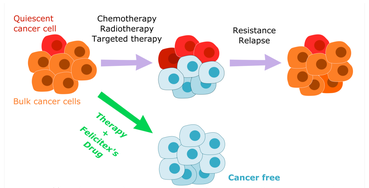
INTRODUCTION

- FX9847 is a selective small molecule ATP competitive inhibitor of DYRK1B kinase, and is a potential treatment in the development for DYRK1B-amplified cancers (such as lung, pancreatic, ovarian, colon, and other cancers[1]), with opportunities to combine with EGFR TKIs or chemotherapies.
- As the emergence of resistance to currently approved EGFR TKIs is seen clinically in NSCLC, we suggest that cancer cell quiescence is a possible mechanism of such acquired resistance.
- DYRK1B regulates stability of proteins that control exit from the G0 phase of the cell cycle[3] and is essential for maintenance of cancer cells in quiescent state, i.e. reversible cell cycle arrest in G0.
- We hypothesized that DYRK1B inhibition would enhance the effects of inhibitors of oncogenic signaling such as EGFR TK inhibitors.
- We demonstrated that the anti-proliferative response of NSCLC with mutant EGFR to EGFR TK inhibitors was partially due to the entry of cells into a quiescent (G0) state associated with an induction of DYRK1B expression. Moreover, addition of FX9847, inhibitor of DYRK1B, reversed the G0 arrest in cells exposed to EGFR TK inhibitors and induced apoptosis.
- Combined therapy of EGFR TKIs and FX9847 significantly enhanced the efficacy of treatment compare to either drug alone.
- We provide emerging preclinical evidence supporting the clinical investigation of combining EGFR TKIs with our DYRK1B inhibitor, FX9847.

BACKGROUND

The problem of quiescent cancer cells (reversibly non-proliferating cells arrested in G0 phase of the cell cycle), a type of cancer dormancy, has been recognized since the 1980s[5]. Cancer cell quiescence is known to be a major mechanism of cancer resistance and recurrence[6].

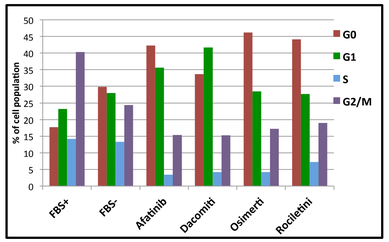


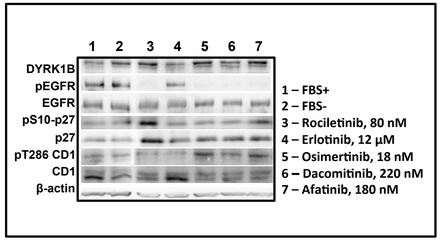
The proportion of cancer cells in quiescent state can increase in response to therapy, producing pharmacological quiescence.
Despite obvious medical need to target quiescent cancer cells, there are only few reports of successful targeting the quiescent cancer cells and there is no anti-quiescent therapy in clinical development.
This is the first report of successful targeting of quiescent cancer cells in lung cancer and NSCLC, specifically.

FX9847 STRUCTURE

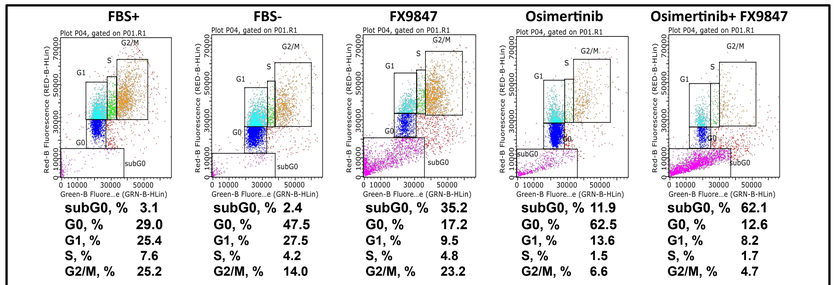
- IC_{50} = 40 nM against recombinant DYRK1B.
- Selective within the kinome (DiscoveRx KINOMEscan™ Profile): 4 out of 450 kinase panel.
- Atypical for anilinoquinazoline binding posture within the ATP binding pocket.
- Designed, optimized, and synthesized in collaboration with Selvita S.A. (Krakow, Poland).

EGFR TK INHIBITORS INDUCE QUIESCENCE IN NSCLC



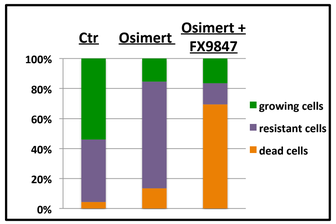


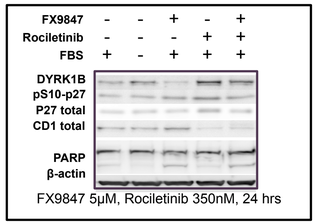
- EGFR TKIs exposure leads to an increase in G0 (quiescent) fraction of cancer cells – a protective niche that confers a survival advantage (NSCLC H1975 cells (L858R, T790M), other tested NSCLC behave similarly).
- Consistent with the quiescence phenotype, DYRK1B expression is induced as well as phosphorylation of its substrates.



H1975 cells were treated for 48 hrs, followed by FACS analysis, AO staining

- Treatment with osimertinib (Tagrisso®) results in an increase of cells in G0 state.
- Combination with FX9847 reversed osimertinib pharmacological quiescence.
- Combination of FX9847 and osimertinib induces apoptosis: 62% vs 12% with osimertinib alone.
- Postulated mechanism is consistent with data on DYRK1B inhibition and phosphorylation of its substrates.





FX9847 5μM, Rociletinib 350nM, 24 hrs

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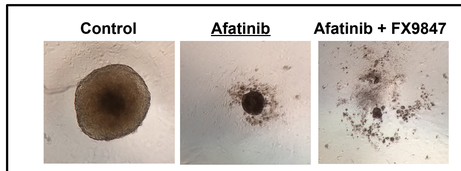
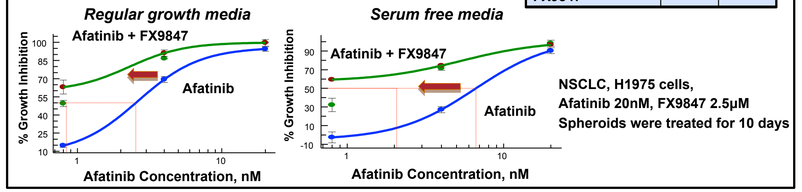
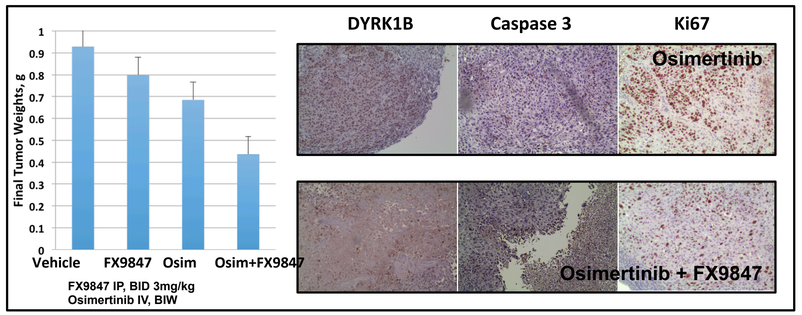
FX9847 IS HIGHLY POTENT IN NSCLC SPHEROIDS



EC50 values, nM	FBS +	FBS -
Afatinib	2.5	6.6
Afatinib + FX9847	<0.2	<0.3
Osimertinib	0.68	3.3
Osimertinib + FX9847	0.28	0.96



IN VIVO EFFICACY IN NSCLC



CONCLUSIONS

- EGFR TKIs as a therapeutic class cause pharmacological quiescence; 1st, 2nd, and 3rd generation EGFR TKIs, reversible or irreversible, anilinoquinazoline- or anilinopyrimidine-based, drive cancer cells into G0 (quiescent) state of the cell cycle.
- Combination of EGFR TKI with FX9847, DYRK1B inhibitor, reverses the pharmacological quiescence and dramatically enhances the lethality of EGFR inhibition *in vitro* and *in vivo.*
- PD markers observed *in vivo* are consistent with the proposed MOA for FX9847.
- Combination of DYRK1B inhibitor, FX9847, with any EGFR TKI has the potential to improve clinical outcomes in patients with NSCLC.