

# Activity of Savolitinib against MET Ex14 mutations and resistance to METi through decoupling from MYC expression

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## Abstract

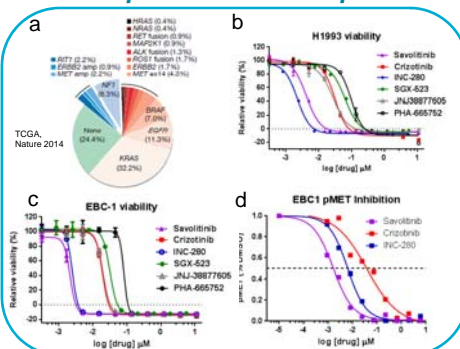
Alterations in the MET oncogene occurs across a broad range of tumor indications. Amplification or mutations in MET lead to increased activity of downstream pathways including PI3K and MAPK, eventually resulting in tumor formation. Several small molecule inhibitors are currently in clinical trials, including the selective inhibitor Savolitinib (HMP-504, Volitinib, AZD6094), which shows single digit nanomolar activity in MET-amplified cell lines. Newly emerging data suggest mutations in MET causing complete skipping of Exon 14 occur in approximately 4% of non-small cell lung cancer (NSCLC), and are more rare in other indications [1, 2]. Exon 14 harbors the CBL binding site (Y1003), which is critical for receptor degradation after binding of its ligand, HGF, and suppression of downstream signaling. Clinical trial results with less potent, pan RTK inhibitors Crizotinib (31nM GI50 vs 3nM for Savolitinib) and Cabozantinib show promising early results, but fall short in long term responses. Therefore, better therapies targeting MET are needed. We utilized engineered, as well as endogenously expressing MET Ex14 mutant models to determine activity of Savolitinib. We found that Savolitinib potently inhibited phospho-MET in both model types. In addition, we found that savolitinib inhibited HGF-induced growth of the NSCLC model H596, which harbors loss of exon 14. In addition to the MET exon14 patient population, MET amplification also drives tumor formation in EGFR WT NSCLC. We previously presented EGFR activation as a resistance mechanism to Savolitinib in some clonal subpopulations [4]. Here, we present an underlying mechanism of resistance found in all clones tested. We found that decoupling of MYC expression from MET activity was a hallmark of resistance. Overexpression of MYC in parental H1993 cells in a doxycycline-dependent manner resulted in resistance to Savolitinib. Parental as well as Savolitinib resistant H1993s depended on MYC expression, as knockdown resulted in loss of viability. Together, this data demonstrates that Savolitinib is active against clinically relevant MET Ex14 mutations in addition to amplification, and that resistance ultimately may develop through decoupling MYC activity from MET signaling.

## Introduction

- MET is altered by either amplification (2%) or Ex14 mutations (4%) in patients with EGFR WT, NSCLC.
- Savolitinib (selective MET inhibitor) is more potent than Crizotinib (multikinase inhibitor) at targeting Ex14 mutations, as well as amplified MET.
- In H1993 clones (MET amp) generated to be insensitive to Savolitinib, resistance can be acquired through decoupling from MYC expression.
- Combinations that target MET as well as MYC may provide a mechanism to overcome resistance.

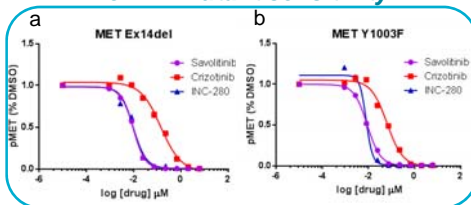
## Results

### METi comparison in Met Amp NSCLC

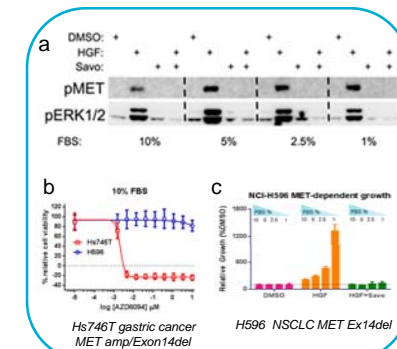


**Figure 2.** Prevalence of genomic alterations in NSCLC and head to head comparison of Savolitinib to other known MET inhibitors (METi).

### Exon 14 mutant sensitivity

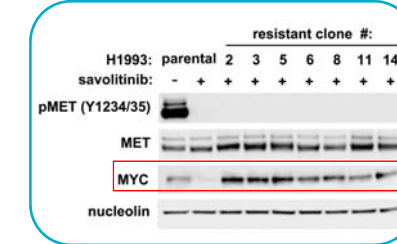


### H596 (Ex14del) and Hs746T (Ex14del/Amp) respond to Savolitinib



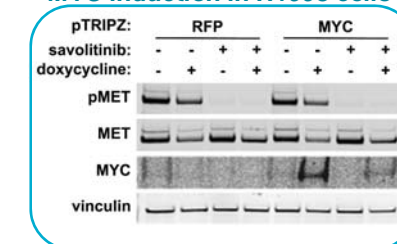
**Figure 3.** Exon14del model sensitivity to Savolitinib with (H596) and without (Hs746T) HGF induction.

### Savolitinib resistance via MYC



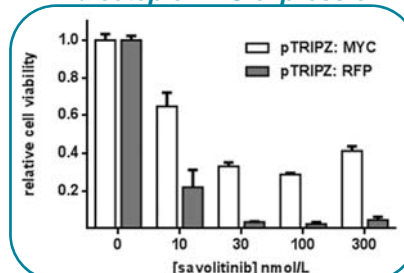
**Figure 4.** MYC expression is increased and decoupled from MET activity in Savolitinib resistant H1993 cells. Resistance was generated through serial passage in the presence of compound.

### MYC induction in H1993 cells



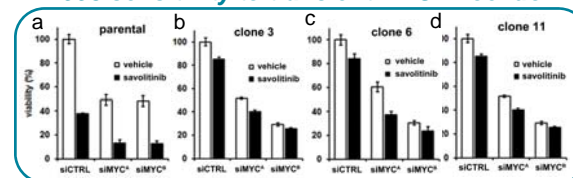
**Figure 5.** Dox-inducible MYC expression in H1993 model.

### Engineered Savolitinib resistance via ectopic MYC expression



**Figure 6.** Induction of MYC in H1993 cells causes resistance to Savolitinib.

### H1993 sensitivity to transient MYC knockdown



**Figure 7.** Savolitinib resistant clones retain dependency on MYC.

## Conclusions

- Savolitinib is a more potent inhibitor of MET ex14 mutations and amplifications than Crizotinib.
- Resistance to Savolitinib in H1993 cells is caused by a common mechanism through loss of MET coupling to MYC expression. This is consistent with a recent publication linking MYC and MET (5)
- Resistant clones still depend on MYC, therefore combinations with agents that reduce MYC expression may aid in combating resistance.

## References

1. Paik, P.K., et al., Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. *Cancer Discov.* 2015. 5(8): p. 842-9.
2. Frampton, G.M., et al., Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Discov.* 2015. 5(8): p. 850-9.
3. Cancer Genome Atlas Research, N., Comprehensive molecular profiling of lung adenocarcinoma. *Nature.* 2014. 511(7511): p. 543-50.
4. D'Cruz et al. 2015 Preclinical data for changing the paradigm of treating drug resistance in NSCLC: Novel combinations of AZD6094, a selective MET inhibitor, and AZD2921 an irreversible, selective (EGFR and T790M) EGFR TKI. *AACR 2015 Poster*

**Figure 2.** Dose response curves of Savolitinib head to head with Crizotinib and INC-280 in transiently transfected 293T cells

	Cell Line	Savolitinib	INC280	Crizotinib
Viability IC <sub>50</sub>	H1993	0.004	0.002	0.031
	EBC1	0.002	0.002	0.019
pMET IC <sub>50</sub>	EBC1	0.001	0.006	0.039
	293T MET (WT)	0.007	0.006	0.079
	293T MET (Y1003F)	0.008	0.007	0.063
	293T MET (Ex14del)	0.009	0.009	0.140

**Table 1.** Summary of inhibitor potencies. Data quantified using Mesoscale multiplexed assay.