Validation of MET exon 14 skipping mutation as an oncogene

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박소정

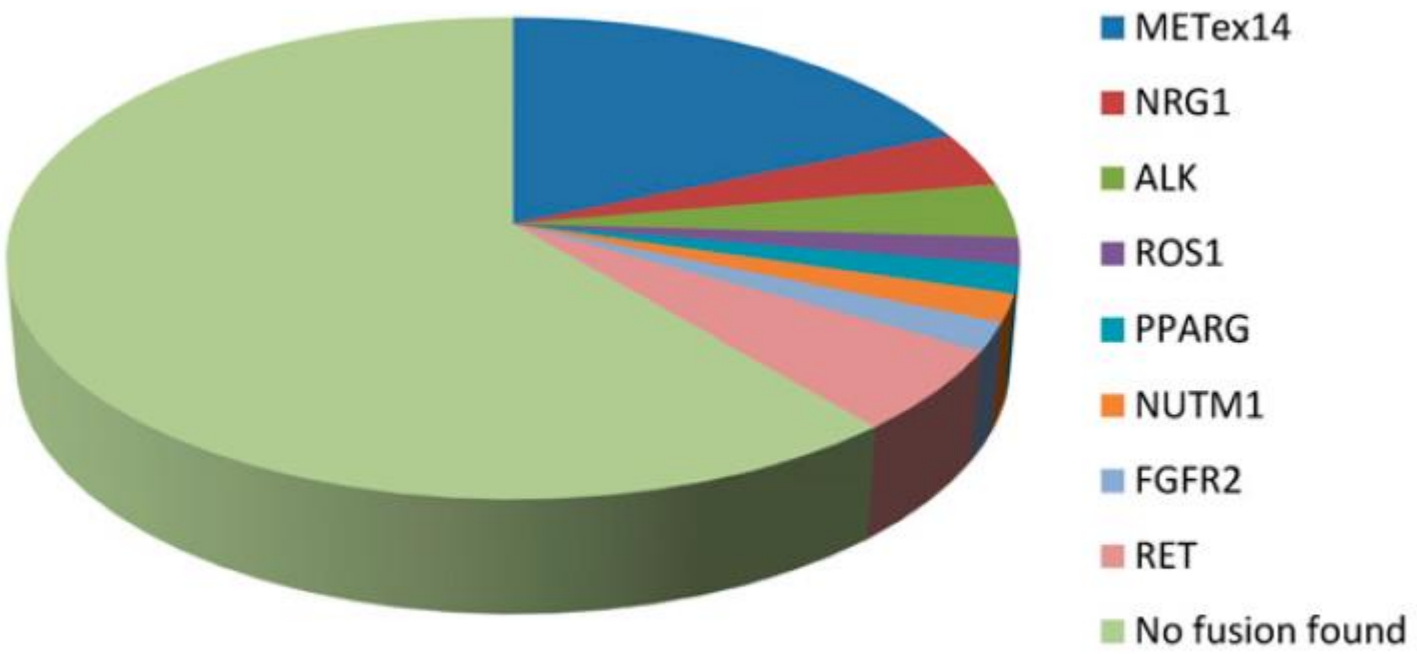


Figure 1. Rearrangements identified among never-smokers with cancers that were wild-type on SNaPshot for known drivers.

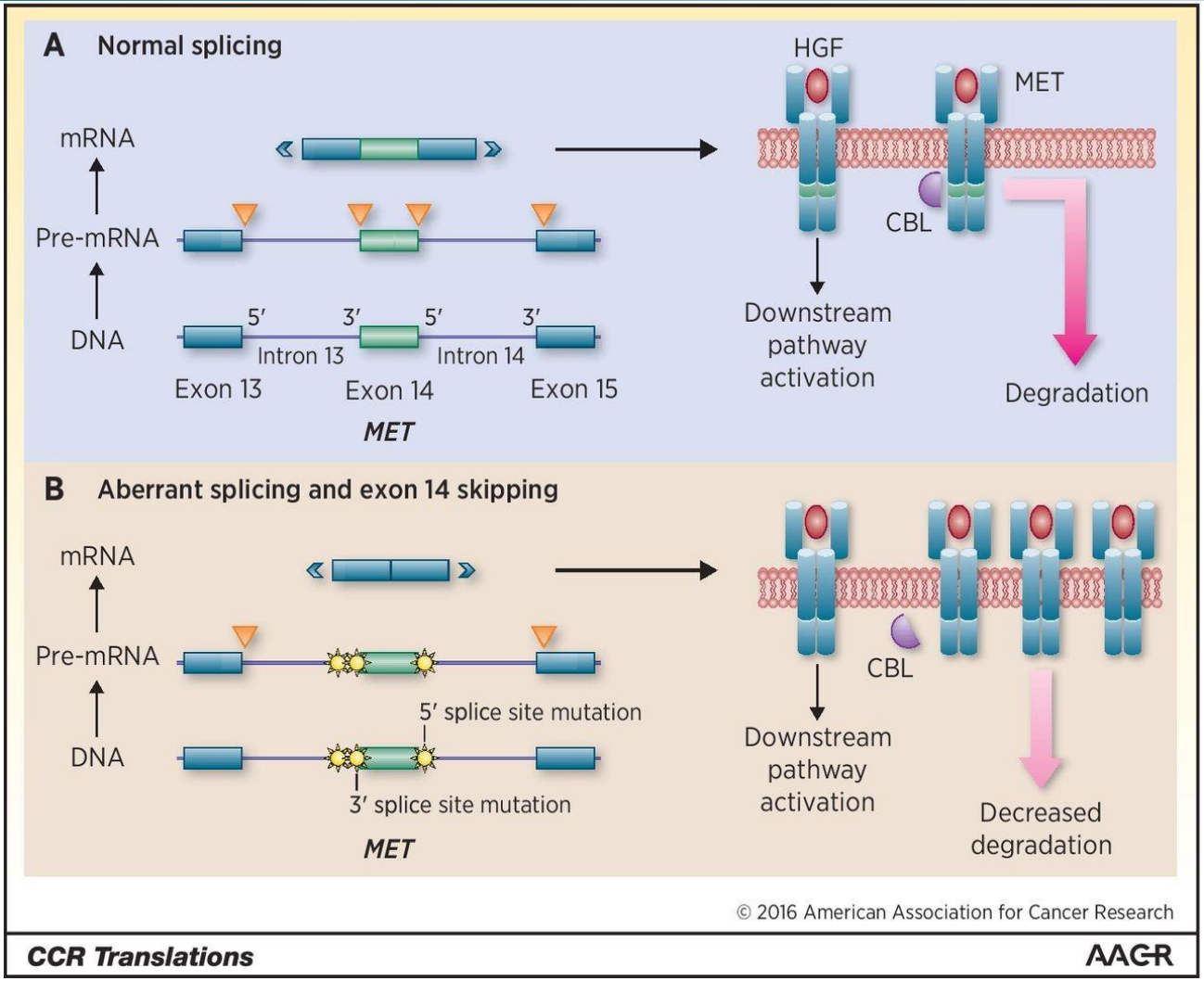
Oncologist 2016;21:481-6

Clinical characteristics

- Met exon 14 skipping prevalence ~3-4% of NSCLC patients population
- MET exon 14 skipping occurs in older patients, in 50-60% patients with tobacco use
- No overlap with other NSCLC oncogenic drivers

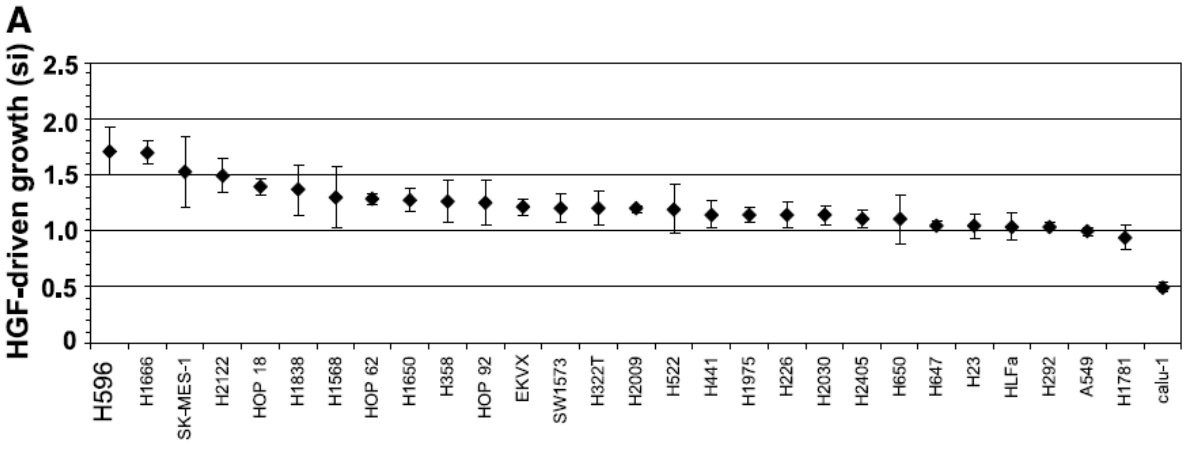
Histologic type	Prevalence
Sarcomatoid	15.3%
Adenosquamous	4.3%
Adenocarcinoma	2.5%
Squamous cell	1.8%

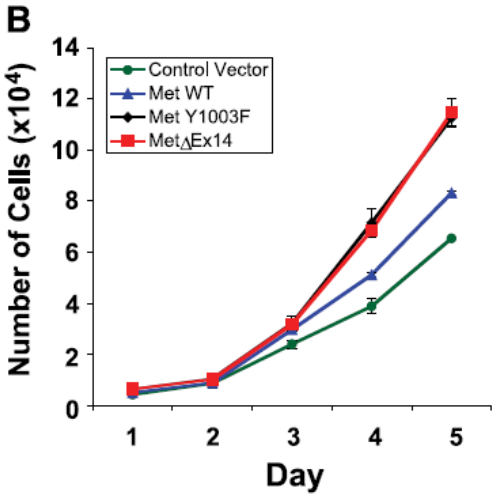
MET exon 14 skipping

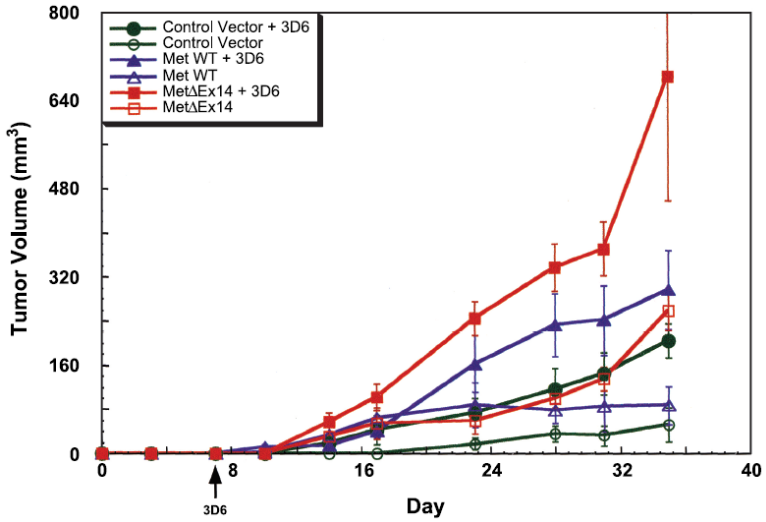


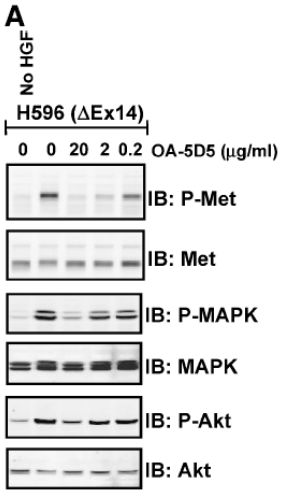
Clin Cancer Res 2016

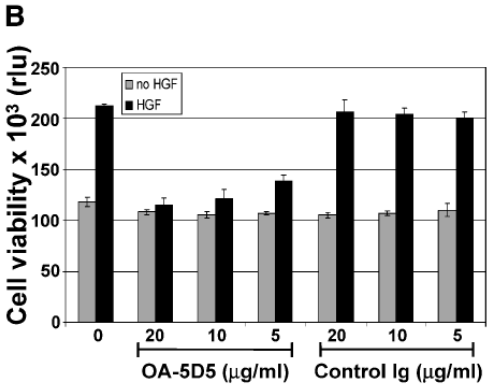
MET Exon 14 skipping







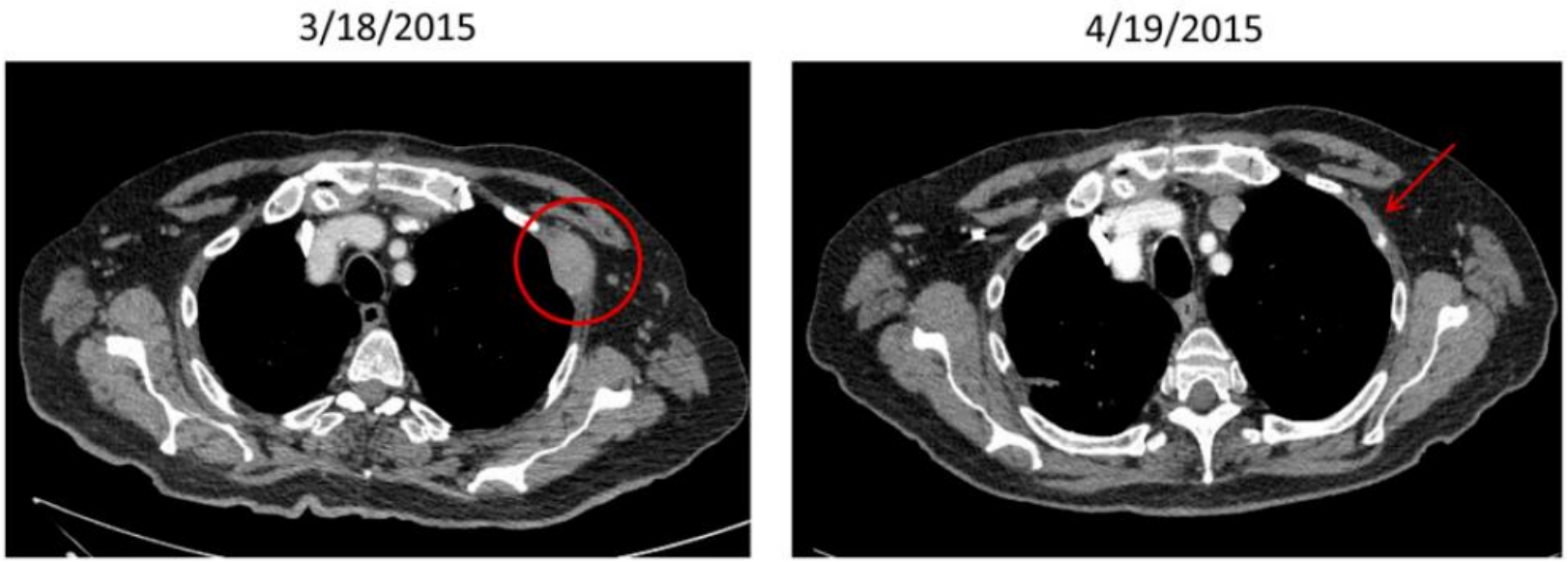




Cancer Res 2006;66:283-9

Case Vignette

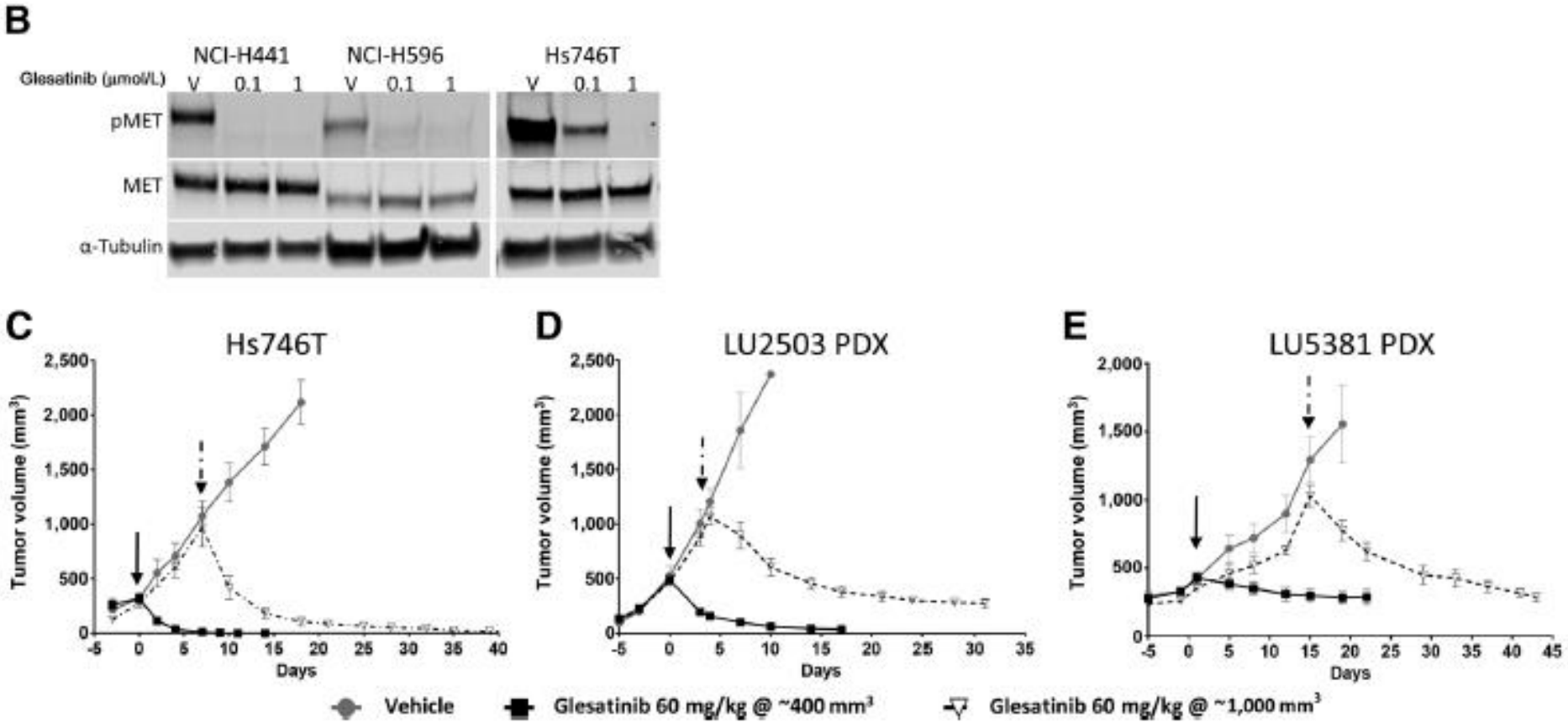
A 73-year-old man with metastatic squamous cell lung cancer
MET exon 14 skipping +
After 4 weeks of crizotinib



Oncologist 2016;21:481-6

Glesatinib

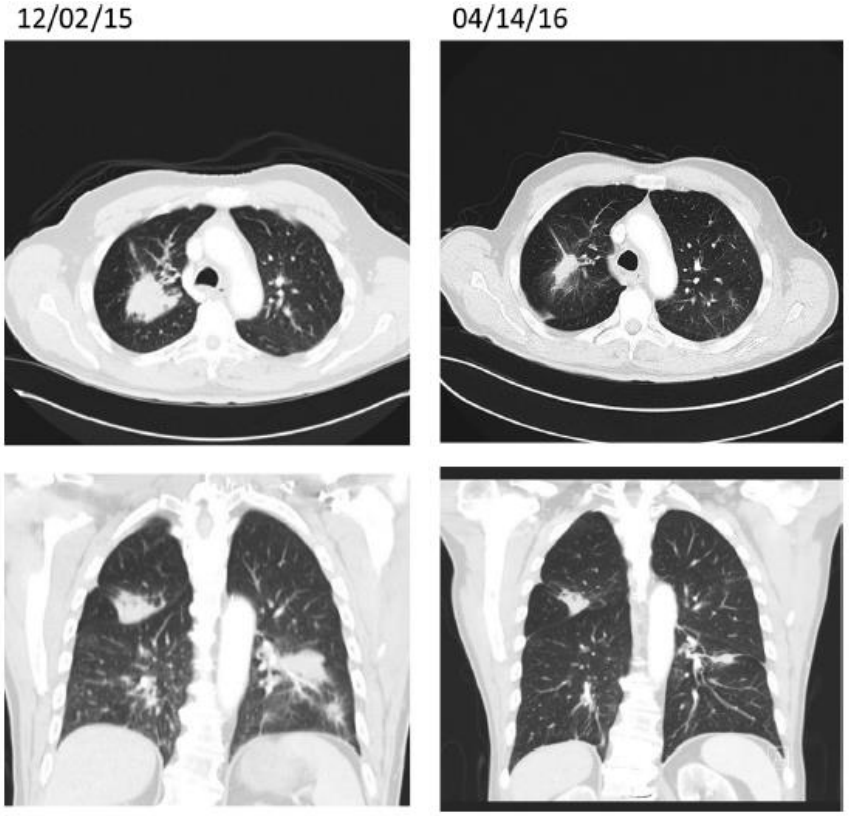
Acquired resistance after crizotinib, D1228N/H and Y1230H mutations in exon 19..



Clin Cancer Res 2017;23:6661-72

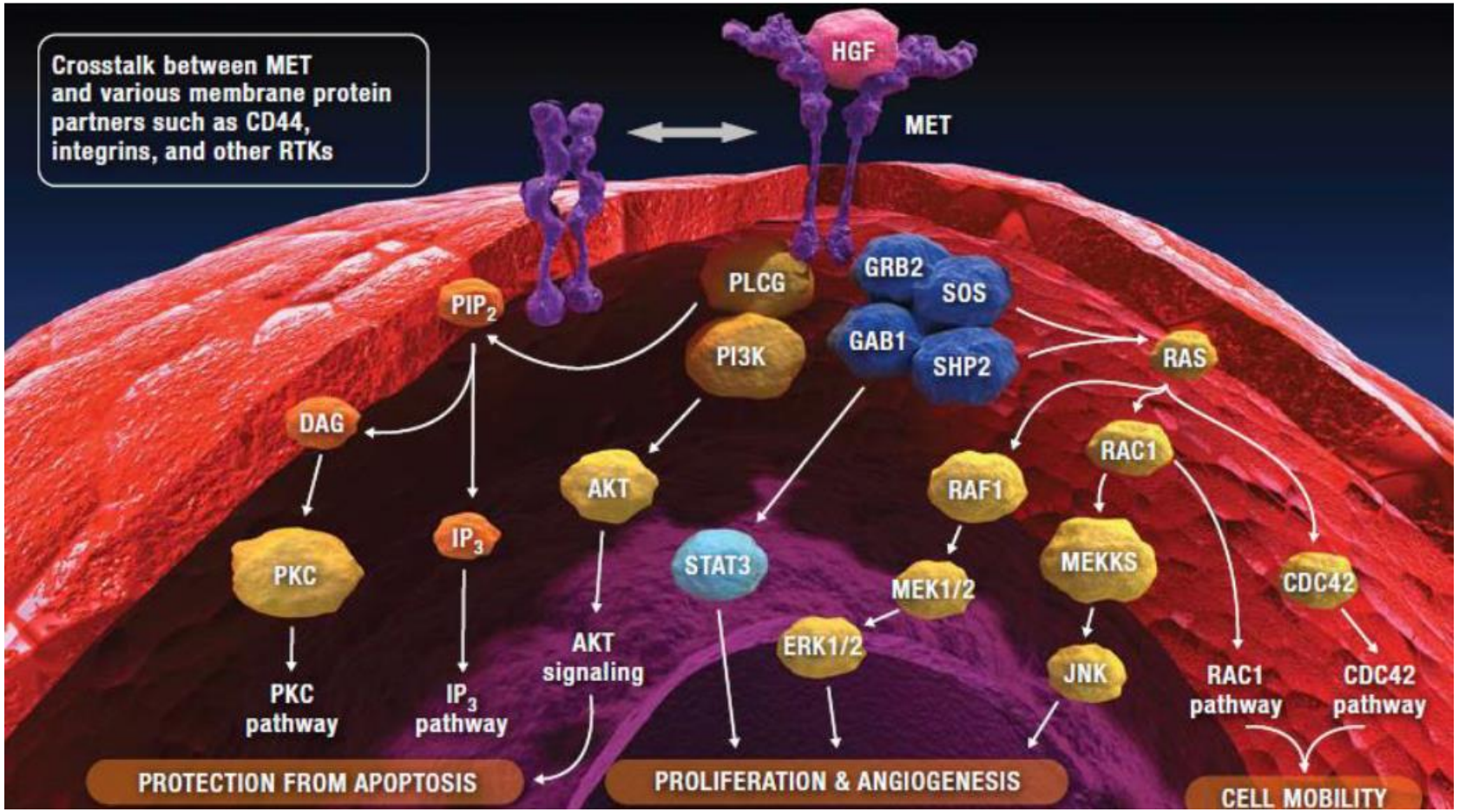
Glesatinib

A 70-year-old never smoker with metastatic adenocarcinoma
MET exon 14 skipping +, TP53 mutation
After 4 months of glesatinib



Clin Cancer Res 2017;23:6661-72

MET-regulated signaling pathways



Cross-talk between MET and RON

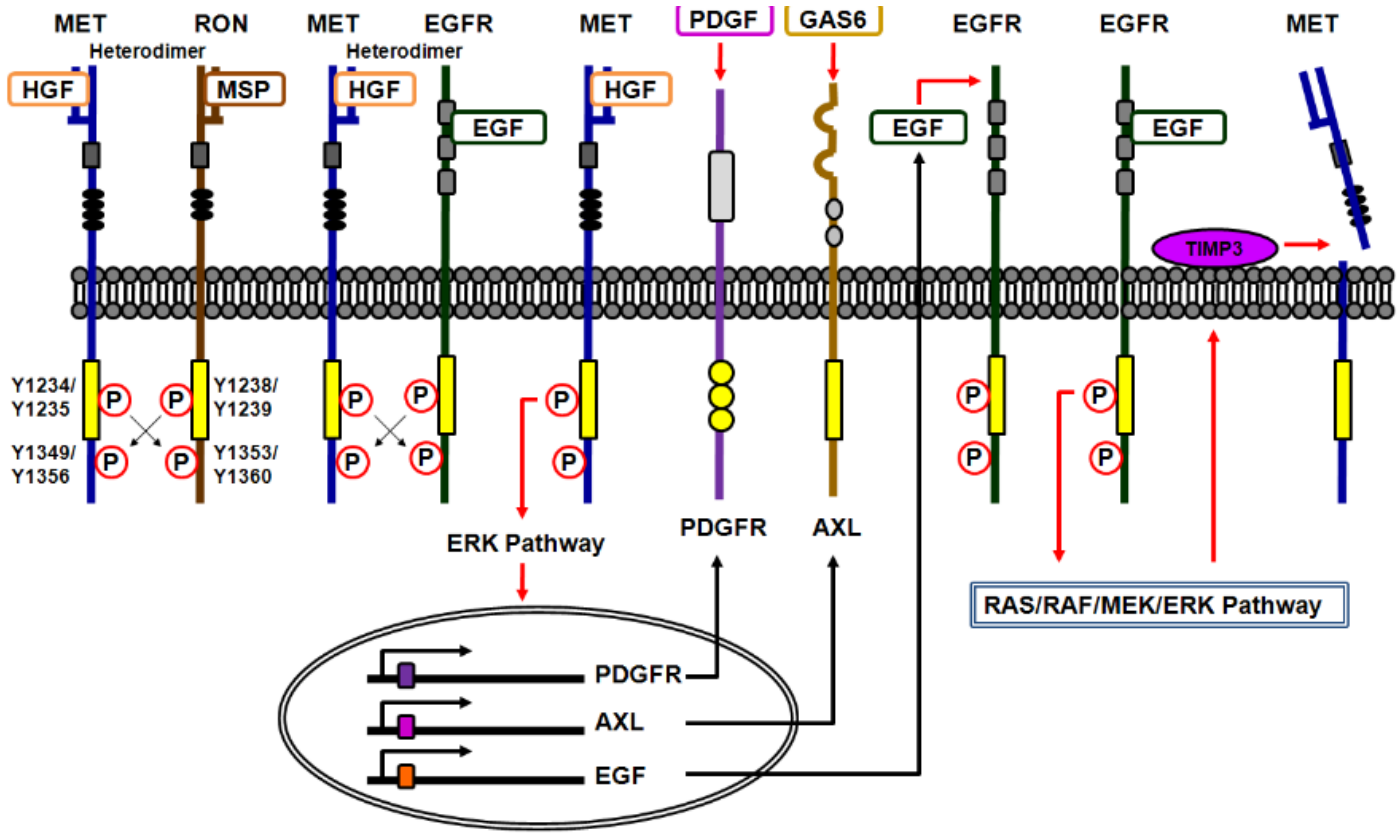
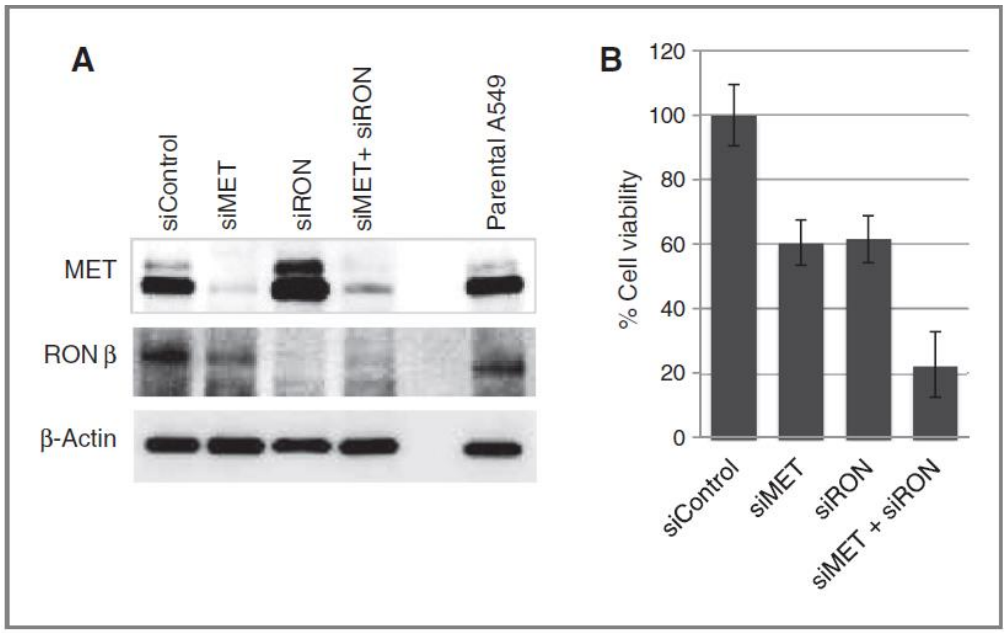
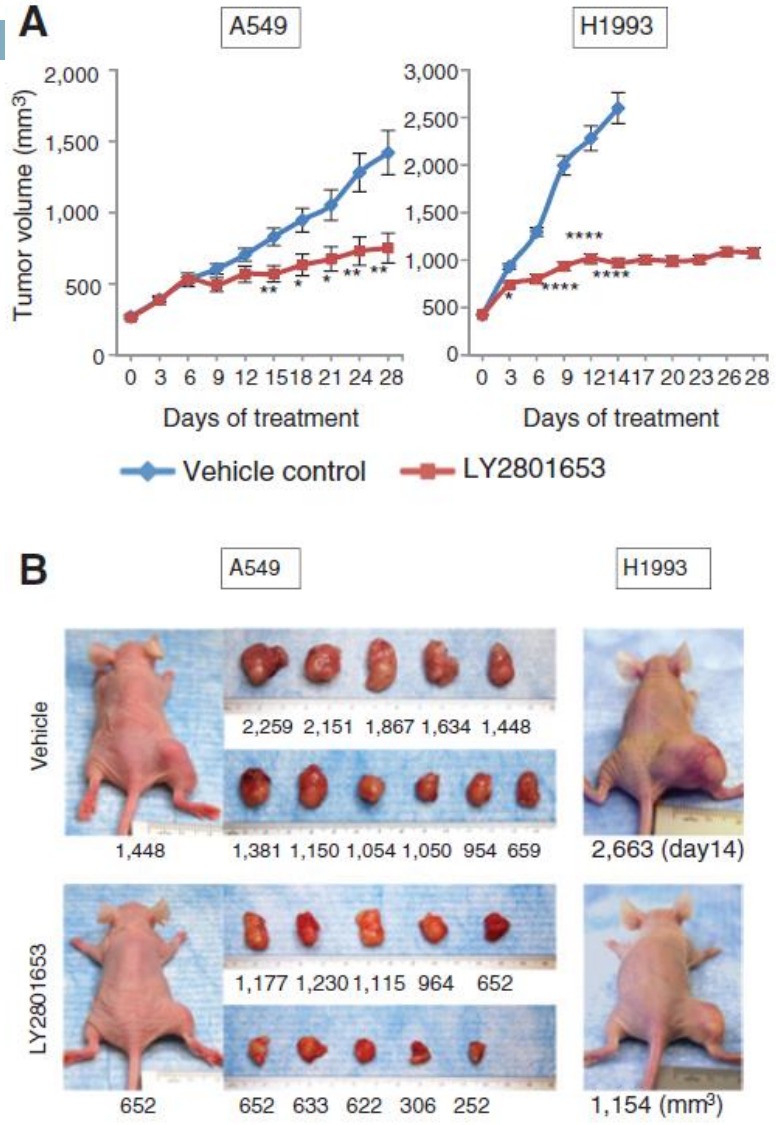


FIGURE 1.

The crosstalk between c-MET and related receptor tyrosine kinases

Merestinib (LY2801653)





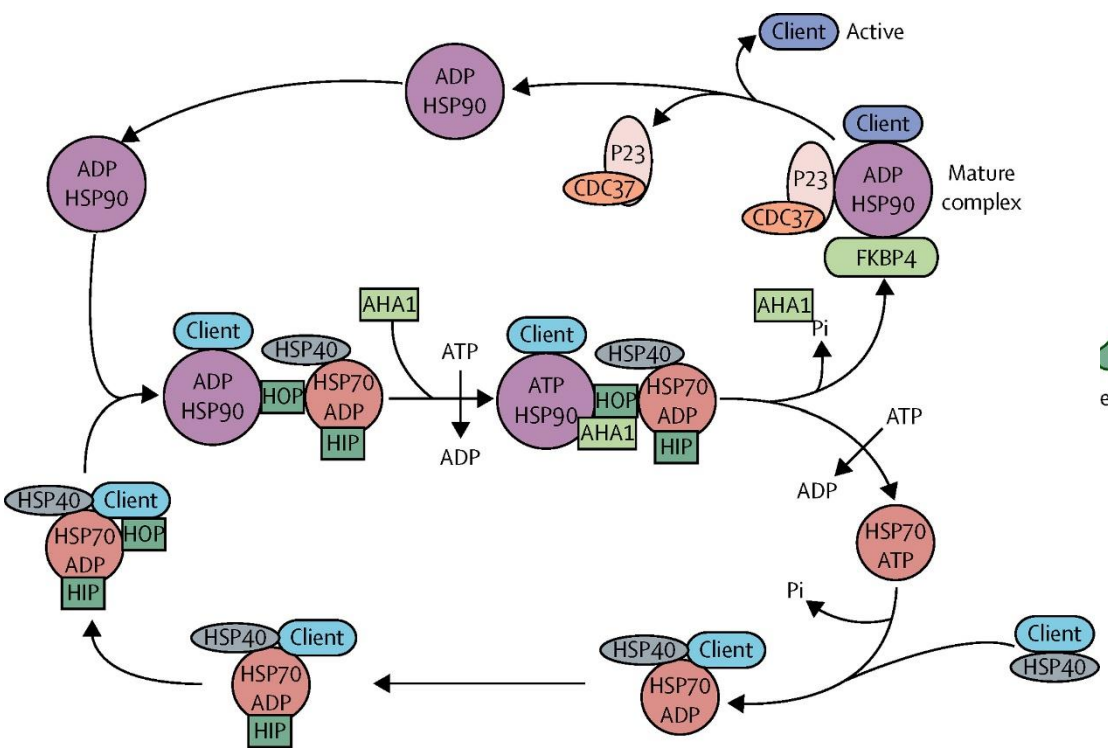
Cancer Res 2014;74:884-95

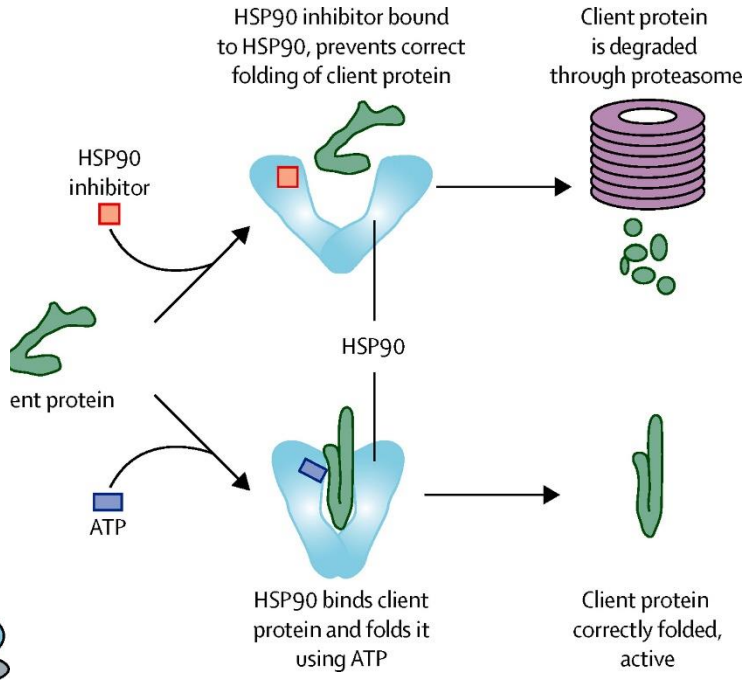
연구 목적

- MET exon 14 skipping mutation 단독 기전으로 암을 유발할 수 있는지 실험적으로 확인된 바가 없음

- MET exon 14 skipping mutation이 암을 유발하는 기전을 실험적으로 확인

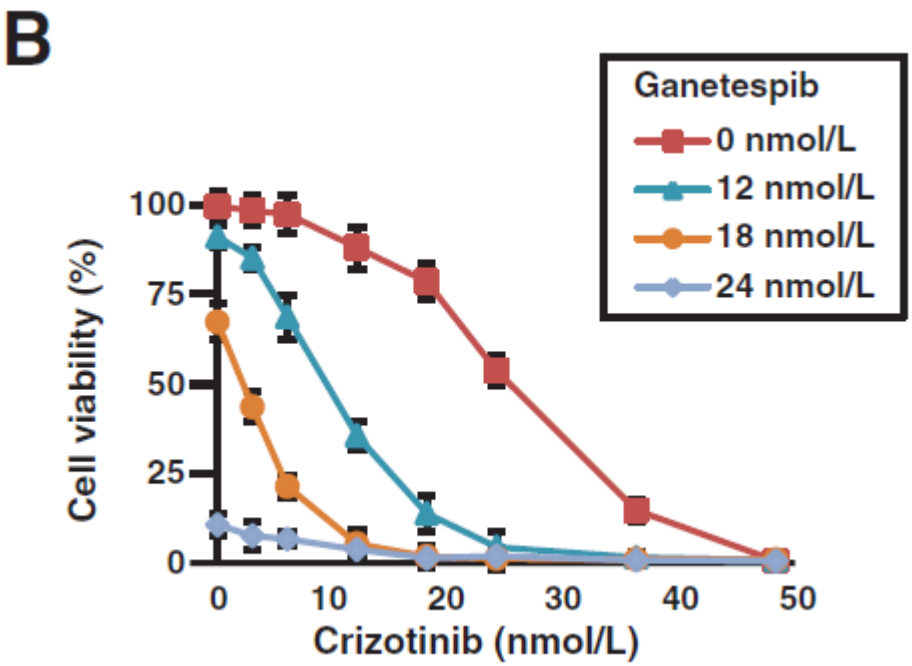
HSP90 inhibitor

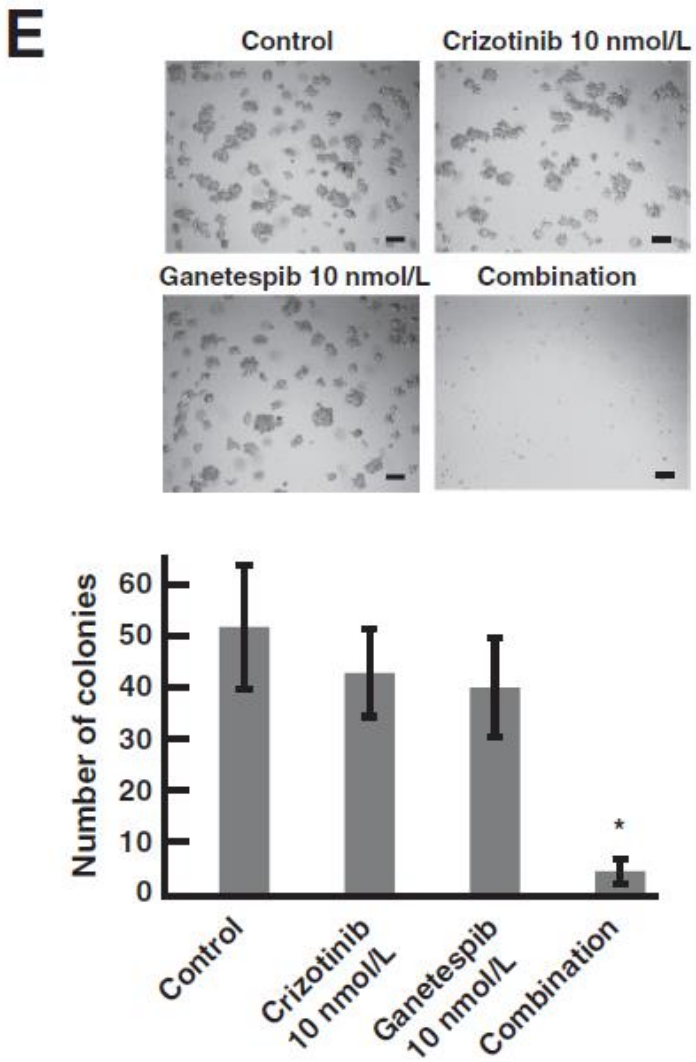




Lancet Oncol 2013;14:e358-69

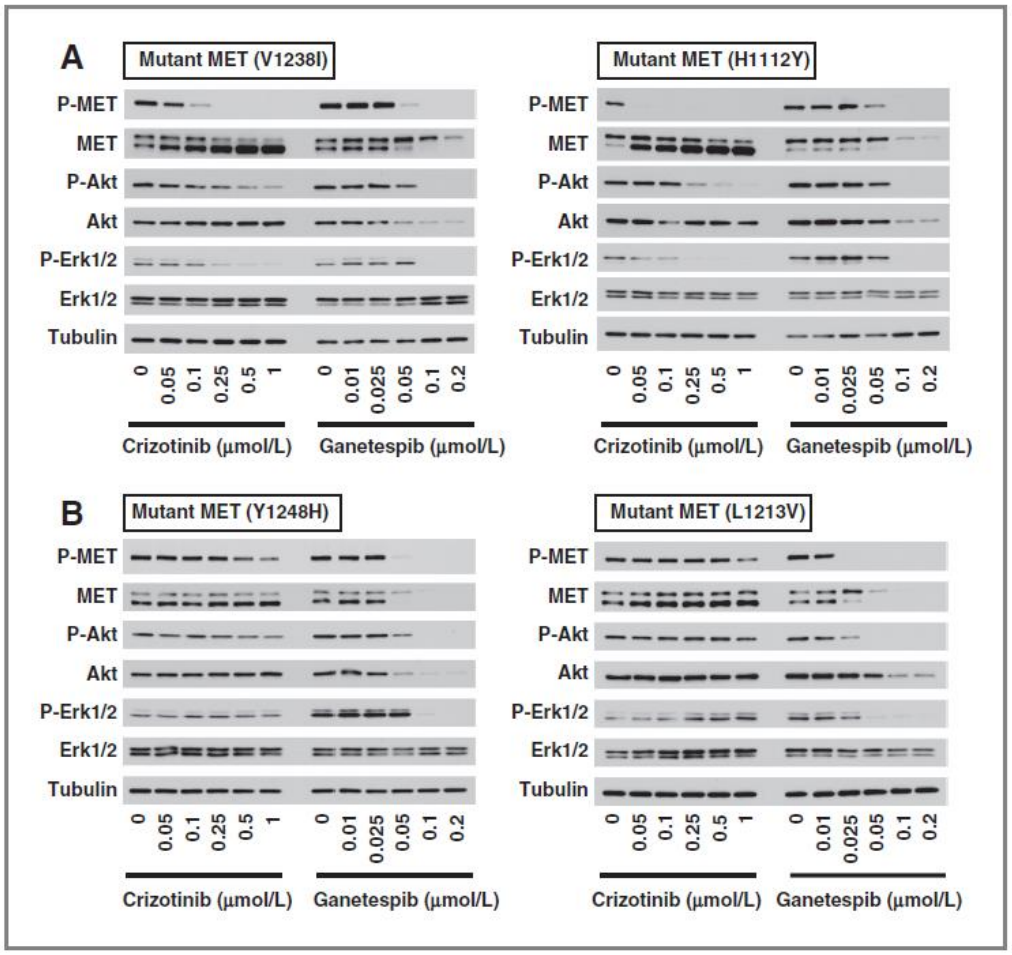
HSP90 inhibitor and crizotinib

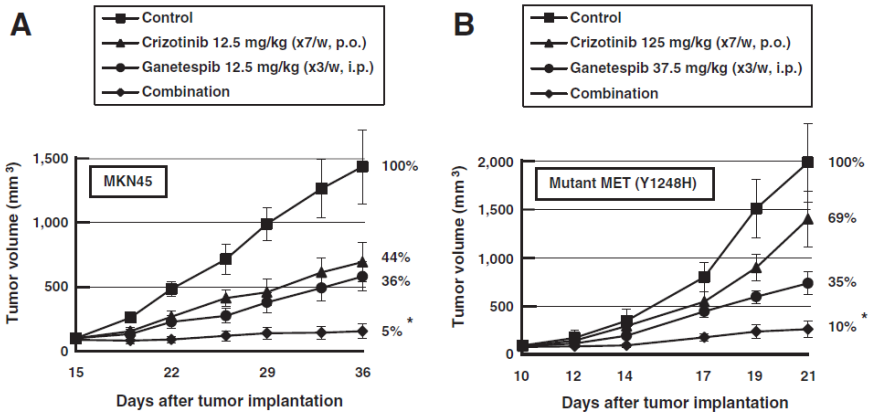




Cancer Res 2013;73:7022-33

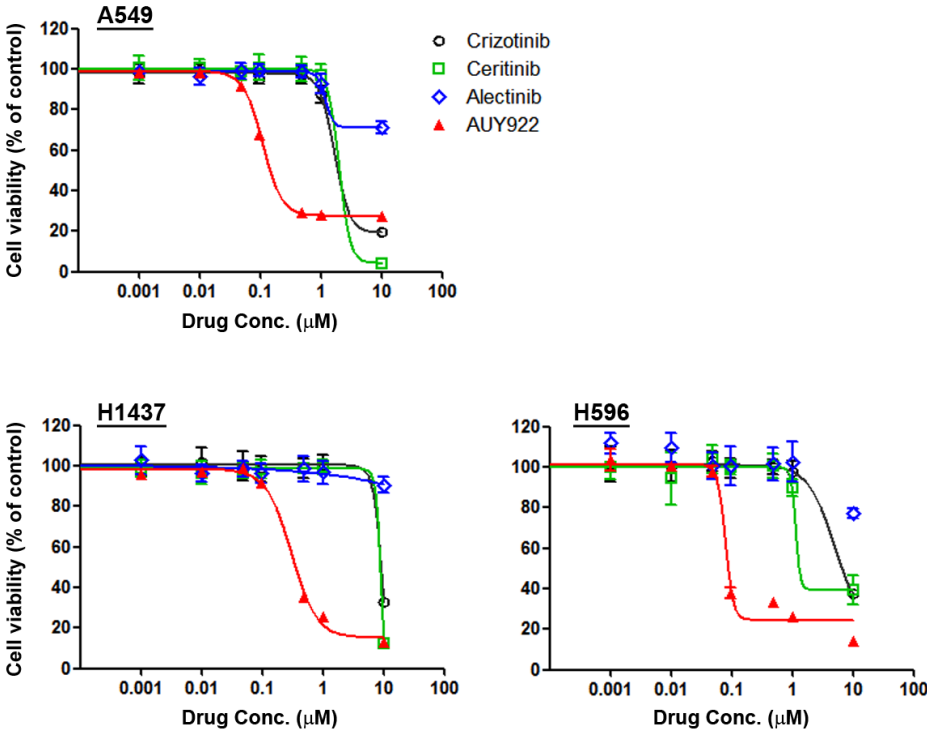
HSP90 inhibitor and crizotinib

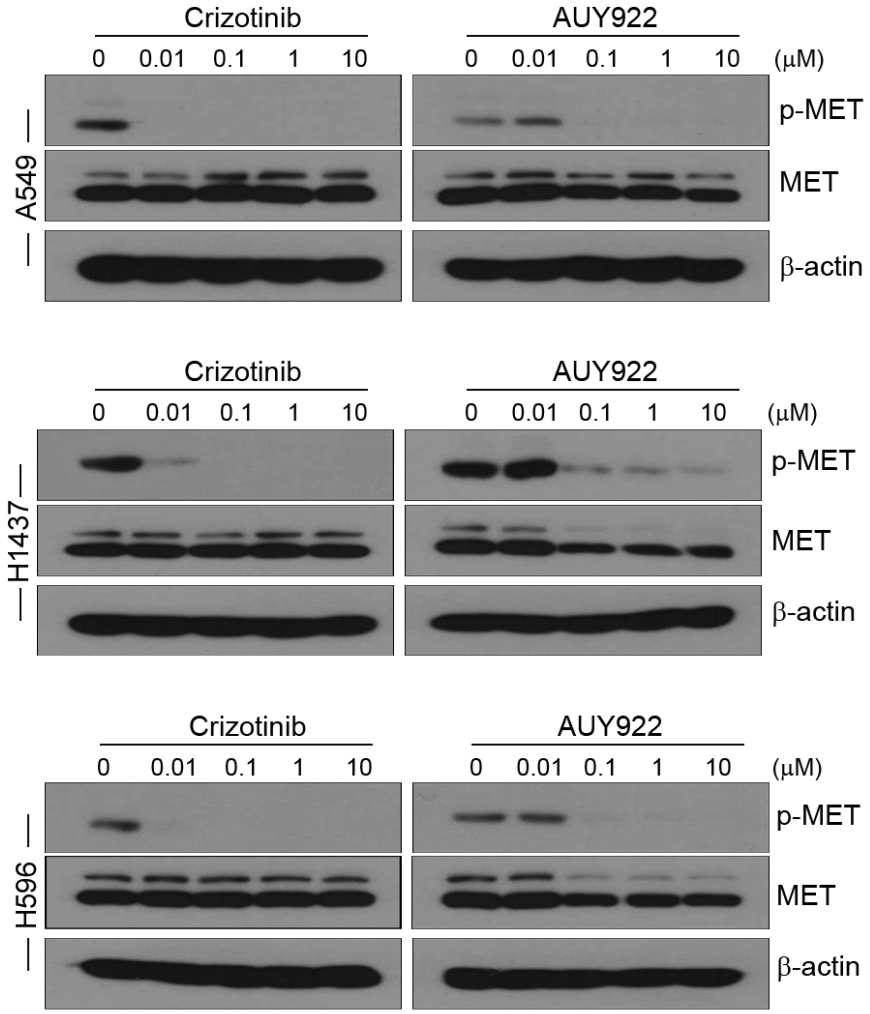




Cancer Res 2013;73:7022-33

Preliminary research





연구 방법

- MET exon 14 skipping mutation cell-line 개발

  - MET exon 14 skipping mutation을 가지고 있는 세포주에서 각각의 mutant MET gene을 cloning 후 sequencing을 통해 확인
  - 각각의 mutant MET gene을 lentiviral vector에 cloning 하고 viral particle 완성
  - 각각의 mutant MET clone은 Ba/F3 세포주에 infection. 이때 stable cell line의 selection은 IL-3 첨가 여부로 결정
  - 각각의 stable cell line을 Nude mice에 injection하여 tumor 생착 여부 확인

연구 방법

- MET inhibitor에 대한 cell viability 변화 및 관련 signaling 변화 확인

- MET exon 14 skipping mutation cell-line과 wild type MET을 가지고 있는 비소세포폐암에서 MET inhibitor (crizotinib, ceritinib, merestinib)의 반응을 MTT assay을 통해 확인

- 각각의 MET inhibitor을 dose dependent하게 처리하였을 때 MET-related signaling (MET, Akt, ERK) 변화를 western blotting으로 관찰

- 각각의 MET inhibitor을 dose dependent하게 처리하였을 때 apoptosis 및 cell cycle변화 관찰

연구 방법

- MET exon 14 skipping mutation 존재 시 MET inhibitor의 항암 반응 결정 인자 발굴 (primary and acquired resistant 인자)

- MET exon 14 skipping mutation을 가지고 있는 세포주에서 MET inhibitor에 반응이 없는 세포주를 활용

- HTS 스크린 시스템을 활용하여 기존 MET inhibitor (0.1 uM)과 잘 알려진 receptor tyrosine kinase inhibitor (0.1 uM)을 combination하여 IC_{20} 이하 값으로 내려가는 표적 발굴

- 기 발견된 표적을 shRNA 혹은 CRISPR/Cas9 시스템을 활용하여 발현을 억제한 후 MET inhibitor의 항암 효과 분석

연구 방법

- MET exon 14 skipping mutation을 가지고 있는 비소세포폐암에서 Hsp90 inhibitor의 활용 방안 도모

- Wild type 및 mutant MET을 가지고 있는 NSCLC cell-line에서 Hsp90 inhibitor인 AUY922의 반응을 MTT assay을 통해 확인

- MET 단백질의 세포내 기능 수행에 있어서 Hsp90의 역할 이해 (wild type Vs. mutant type): client protein의 역할

- 항암 반응 기전 분석 (western blotting, xenograft model 활용)

감사합니다