Date: February 26th, 2019

The summary of doctoral dissertation in lieu of the entire texts of electronic file

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Title of Thesis:

Discovery of a Novel Epidermal Growth Factor Receptor Inhibitor Targeting Exon 20 Insertion Mutations

Summary:

Among cancer-related deaths, deaths from lung cancer are the most common in the world. In NSCLC, somatic mutation of the EGFR is a major oncogenic driver and is present in approximately 30-50% and 10-20% of NSCLC in Asians and in Americans and Western Europeans, respectively. Activating mutations in the EGFR kinase domain lead to cancer cell growth and survival by inducing ligand-independent constitutive activation and subsequent signal trunsduction. Given its important role in cancer, mutant EGFR is a crucial target in lung cancer therapy. Deletion mutations in exon 19 and the L858R substitution mutation in exon 21 are most common somatic EGFR mutations and account for over 80% of EGFR mutations. Preclinical models have shown that these common mutations are sensitive to ATP-mimetic EGFR-tyrosine kinase inhibitors (TKIs). Patients with NSCLC harboring these drug-sensitive mutations show more significant responses to EGFR-TKI therapies, including gefitinib, erlotinib, and afatinib, than to standard chemotherapies.

The next largest proportion of EGFR mutations is a family of exon 20 insertions, which accounts for roughly 4-13% of all EGFR mutations in NSCLC patients; these mutations consist of in-frame insertions of 3-21 base pairs predominantly within the range of codons 762 to 774. These insertions have characteristics of oncogenic driver mutations as well as common mutations. However, in contrast to common mutations, exon 20 insertions do not sensitize the kinase domain to EGFR-TKIs, behaving as intrinsic resistant mutations. Furthermore, patients with NSCLC harboring these mutations exhibit poor clinical responses to monotherapy with afatinib, gefitinib, and erlotinib because plasma concentrations of these drugs in clinical settings are kept low by dose-limiting toxicity caused by wild-type (WT) EGFR inhibition.

It is reasonable to hypothesize that agents, which are able to inhibit exon 20 insertion mutant EGFR while sparing WT EGFR, might achieve target inhibition in vivo, leading to improvement of the

clinical issue on therapy for these patients.

To identify small molecule compound which has the profile above, compound screening have been conducted with the kinase inhibitor library. As a result of biochemical assay, we have identified TAS6417, a novel EGFR-TKI with a unique scaffold, showing potent inhibitory activity against human recombinant EGFR and its mutations. Binding mode analysis revealed that TAS6417 covalently bound to cysteine at position of 797 in EGFR D770_N771insNPG, an exon 20 insertion mutation, and exhibited irreversible or slow-dissociation kinetics. Furthermore, in a panel of 254 kinases except for EGFR, TAS6417 showed potent inhibitory activity against only six kinases (TXK, BMX, BTK, HER4, TEC, JAK3) whose ATP-binding pockets harbor a cysteine at a structural position homologous to that in EGFR, suggesting high selectivity against EGFR over other kinases.
As a next step, we evaluated cellular potency of TAS6417 in a panel of genetically engineered cell lines expressing human EGFRs. While TAS6417 had a potent inhibitory effect on auto-phosphorylation and cell proliferation driven by mutant-EGFRs including exon 20 insertion mutations, its inhibitory effect on WT EGFR was moderate, indicating mutant-EGFR selective characteristics. This selectivity against exon 20 insertion mutations over WT for TAS6417 was superior to that for other EGFR-TKIs. These findings demonstrate that TAS6417 is a potent and selective kinase inhibitor targeting mutant EGFR including exon 20 insertion mutations, implying the possibility for a novel anti-cancer agent for NSCLC driven by EGFR exon 20 insertion mutations.

Next, the possibility of TAS6417 as a therapeutic agent for NSCLC with EGFR exon 20 insertion mutations was investigated. Since there are few cancer cell lines available as a tool, we established a NSCLC cell line harboring EGFR exon 20 insertion mutation by using gene editing technique. In a cell panel of human NSCLC and keratinocytes, TAS6417 exhibited more potent inhibitory activity of cell growth in human lung cancer cells with EGFR exon 20 insertion mutations than in primary keratinocytes of which WT EGFR is implicated in the growth and survival. Among the EGFR-TKIs tested, the mutation selectivity of TAS6417 was the highest. Furthermore, TAS6417 exerted a potency to inhibit cell growth in NSCLC cell lines with EGFR mutations other than exon 20 insertion mutations, but not in NCI-H23 cells and NCI-H460 cells, of which cell growth independent on EGFR, supporting kinase selectivity of TAS6417 indicated in biochemical assay.
As for biological mode of action, TAS6417 exposure resulted in a concentration-dependent decrease of phospho-EGFR and its downstream molecules, including ATK and ERK, in cell lines driven by EGFR exon 20 insertions. Accordingly, TAS6417 led to increased levels of Bim , cleaved PARP, and caspase 3/7 activity, implying induction of apoptosis. These findings demonstrate that TAS6417 inhibits EGFR signal transduction, leading to cell growth inhibition and apoptosis induction in NSCLC cells harboring EGFR exon 20 insertion mutations.

Consistent with in vitro potency and selectivity, TAS6417 achieved remarkable and durable inhibition of mutant EGFR and its downstream effectors in mouse xenograft tumors, while sparing WT EGFR in mouse skin tissues. These pharmacodynamic changes correlated well with plasma concentrations. This mutation-selective characteristic led to significant in vivo antitumor activity in mouse and rat models with NCI-H1975 EGFR D770_N771insSVD cell line. Notably, once-daily oral administration of TAS6417 at 100 mg/kg achieved persistent tumor regression with good tolerability in a PDX model of EGFR exon 20 insertions, including V769_D770insASV and H773_V774insNPH. In contrast, afatinib induced tumor growth inhibition, but not tumor regression, and some toxic signatures including body weight loss over 10% and skin symptoms. In addition, a lung orthotropic implantation model in mice also demonstrated the survival benefits of TAS6417, with tolerability in long-term daily administration. These findings demonstrated that mutation selective characteristics of TAS6417 improved antitumor efficacy and tolerability compared to none-mutant selective EGFR-TKIs such as afatinib, supporting clinical evaluation of TAS6417 as an efficacious drug candidate for patients with NSCLC driven by EGFR exon 20 insertion mutations.

TAS6417 was further evaluated for its potency and selectivity against uncommon mutations such as G719X and L861Q that exhibits a mixed response to EGFR-TKI therapy in clinical settings.
Compared to the representative EGFR-TKIs, TAS6417 exerted comparable or higher cellular potency against all EGFR mutations tested, implying a unique mutant-EGFR inhibitory spectrum. Besides potency, the selectivity of TAS6417 against uncommon mutations over WT was the highest among tested EGFR-TKIs.
This potent and mutation selective characteristics of TAS6417 led to significant in vivo antitumor activity superior to afatinib. In NIH/3T3 EGFR G719A model, TAS6417 achieved marked tumor regression at higher doses (100 mg/kg and 200 mg/kg), but afatinib not at its MTD, without severe body weight loss. Similar result was observed in NIH/3T3 expressing EGFR carrying G719A mutation as well as T790M acquired resistant mutation, suggesting TAS6417 may more efficacious than afatinib against the tumors driven by EGFR G719A uncommon mutation.

In summary, we have identified TAS6417 as a novel EGFR-TKI that exerts mutant-selective inhibition against uncommon EGFR mutations including exon 20 insertion mutations, G719X, and L861Q while sparing WT. This mutation-selective characteristic led to a significant antitumor efficacy in animal models, suggesting that TAS6417 may be a promising therapeutic option for NSCLC patients with uncommon EGFR mutations, which results in an increase of QOL for cancer patients. Furthermore, the discovery of this unique scaffold and its varied mutation inhibitory spectrum may produce new research field of EGFR-TKI development, leading to a novel value in the society.