ABSTRACT #1522

THE CDK4/6 INHIBITOR, G1T38, ENHANCES RESPONSE TO TARGETED THERAPIES IN PRECLINICAL MODELS OF NON-SMALL CELL LUNG CANCER

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BACKGROUND

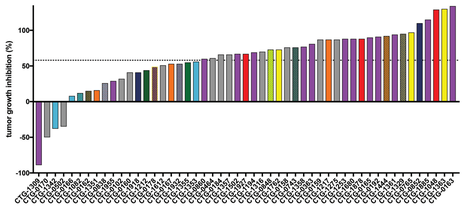
- G1T38 is an oral, potent, and selective small-molecule cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitor in clinical development (NCT02983071; NCT03455829). Preclinical and early clinical data have demonstrated that G1T38 has a differentiated and potentially best-in-class profile based on its ability to be dosed continuously without causing dose-limiting neutropenia, a feature that may result in better anti-tumor efficacy.
- The recent FDA approvals of palbociclib, ribociclib, and abemaciclib in breast cancer validate CDK4/6 as key therapeutic targets and provide strong rationale to investigate CDK4/6 inhibitors in other CDK4/6 dependent tumor types, including non-small cell lung cancer (NSCLC).
- NSCLC accounts for 80-85% of all lung cancers and frequently exhibits oncogenic alterations in KRAS, EGFR, BRAF, ALK, or HER2 genes. Kinase inhibitors targeting these oncogenes (or their signaling pathways) have demonstrated clinical benefit, however, resistance to these agents invariably develops, thus requiring new approaches. One strategy to extend the duration of response and potentiate efficacy involves the inhibition of multiple signaling pathways through targeted therapy combinations. In NSCLC, many of the oncogenic pathways converge at CDK4/6, providing rationale for the use of G1T38 as a backbone for combination therapy in NSCLC.
- Here, we assessed the efficacy of G1T38 as a single agent and in combination with other targeted therapies in NSCLC models harboring common driver mutations providing a rationale for potential combinations in the clinic.

OBJECTIVES

- Assess the efficacy of G1T38 alone and in combination with kinase inhibitors in NSCLCs harboring specific driver mutations in the KRAS, EGFR, BRAF, ALK, HER2, MET, ROS1, and RET oncogenes.
- Evaluate the role for G1T38 in augmenting primary response or delaying/reversing acquired resistance to agents targeting KRAS^{mut}, EGFR^{mut}, and ALK fusions in NSCLC.

RESULTS

FIGURE 1. A PANEL OF NSCLC PDX MODELS DEMONSTRATES VARYING SENSITIVITY TO G1T38 TREATMENT



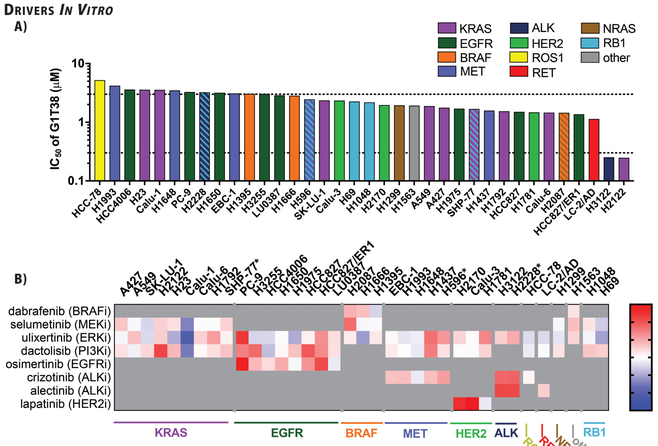
Histology	Median TGI
Adenocarcinoma	73%
Squamous Cell Carcinoma	66%

Genetic Alterations: HER2, KRAS, RB1, RET, ALK, BRAF, ROS, EGFR, other

NSCLC PDX models (n=60) were treated for up to 28 days with daily oral doses of vehicle or G1T38 100 mg/kg (Champions Oncology, Rockville, MD). Tumor growth inhibition (TGI) was calculated when either tumors reached pre-specified tumor burden or on day 28. Genetic alterations from pre-treated samples were evaluated using FoundationOne (Foundation Medicine, Inc, Cambridge, MA). 58% TGI was used as the responder/non-responder cutoff for correlation analysis (Fios Genomics, Edinburgh, UK).

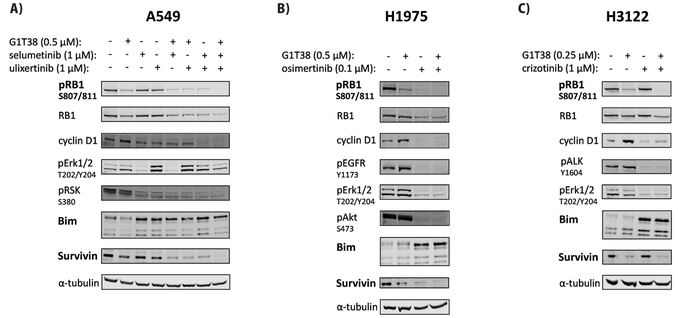
RESULTS

FIGURE 2. G1T38 ENHANCES THE ANTI-PROLIFERATIVE EFFECT OF INHIBITORS TARGETING SPECIFIC ONCOGENIC DRIVERS *IN VITRO*



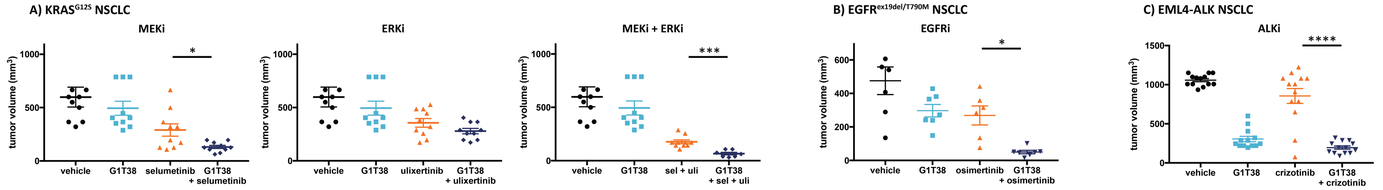
Lung cancer cell lines (n=36) harboring known oncogenic mutations were screened for sensitivity to G1T38 alone or in combination with relevant targeted kinase inhibitors (Crown Bioscience, Taicang, China). (A) Relative IC_{50} values from single-agent G1T38 treatments were calculated using a 2x doubling time cell proliferation assay (minimum 3 days). (B) Relative IC_{50} values from single-agent drug treatments were used to guide the design of the combination treatment assay. Growth inhibition values were used to calculate the synergy scores using the Bliss Independence model. Average Bliss synergy scores for each drug combination were calculated using exclusion criteria that *i)* omitted from the calculation those conditions which produced < 25% inhibition in combination, or *ii)* conditions for which either associated single-agent concentration produced > 90% inhibition. Bliss synergy scores > 5 are indicative of synergy, and scores < -5 represent antagonism. * RB1^{MUT} cell line.

FIGURE 3. G1T38 COMBINATION TREATMENT AUGMENTS THE ANTI-PROLIFERATIVE AND APOPTOTIC SIGNALING PATHWAYS



(A) A549 (KRAS^{G12S} and CDKN2A null), (B) H1975 (EGFR$^{L858R/T790M}$), and (C) H3122 (EML4-ALK fusion) NSCLC cells were treated with G1T38 and/or relevant targeted kinase inhibitors at the indicated concentrations for 48 hours. α-tubulin was used as the loading control.

FIGURE 4. G1T38 ENHANCES THE EFFICACY OF TARGETED THERAPIES IN NSCLC MOUSE MODELS



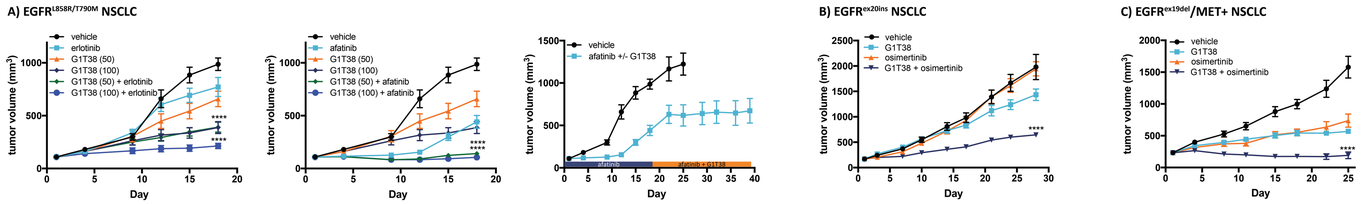
Graphs depict tumor volumes at the end of treatment. Error bars represent SEM. Statistics were completed using a two-tailed Student's t-test comparing targeted agent to targeted agent + G1T38. *p≤0.05, ***p≤0.001, ****p≤0.0001.

(A) A549, a KRAS^{G12S} human NSCLC xenograft model, was treated with G1T38 and/or ERKi/MEKi treatment *in vivo* (Charles River Laboratories, Research Triangle Park, NC). Mice were given once daily doses of vehicle, G1T38 (100 mg/kg), selumetinib (50 mg/kg), or twice daily doses of ulixertinib (50 mg/kg) alone or in combination by oral gavage for 30 days (n=7-10).

(B) TM00219, an EGFR$^{ex19del/T790M}$ NSCLC PDX model, was treated with G1T38 and/or EGFRi treatment *in vivo* (The Jackson Laboratory, Sacramento, CA). Mice were given once daily doses of vehicle, G1T38 (100 mg/kg), or osimertinib (2.5 mg/kg) alone or in combination by oral gavage for 27 days (n=7).

(C) H3122, an EML4-ALK fusion human NSCLC xenograft model, was treated with G1T38 and/or ALK treatment *in vivo* (MI Bioresearch, Ann Arbor, MI). Mice were given once daily doses of vehicle, G1T38 (100 mg/kg), or crizotinib (25 mg/kg for the first 12 days, then 50 mg/kg) alone or in combination by oral gavage for 28 days (n=13).

FIGURE 5. ADDITION OF G1T38 TO EGFRi TREATMENT OVERCOMES RESISTANCE IN EGFR^{MUT} NSCLC



Statistics were completed using linear regression analysis of time during treatment comparing single agent to single agent + G1T38. Error bars represent SEM. ****p≤0.0001.

(A) H1975, a human NSCLC that harbors the EGFR$^{L858R/T709M}$ mutations resulting in primary resistance to erlotinib and secondary resistance to afatinib, was treated with G1T38 in combination with indicated EGFR inhibitors (Charles River Laboratories, Research Triangle Park, NC). Tumor-bearing mice were orally administered daily afatinib (20 mg/kg), erlotinib (70 mg/kg), or G1T38 (50 or 100 mg/kg), as single agents or in combination (G1T38 + erlotinib or G1T38 + afatinib) for the duration of the study (n=10).

(B) LU0387, a NSCLC PDX model that harbors an oncogenic exon 20 insertion in EGFR (H773_V774insNPH) rendering it resistant to EGFR tyrosine kinase inhibitors (TKIs), was treated with G1T38 +/- osimertinib (Crown Bioscience, Beijing, China). Tumor-bearing mice were given once daily doses of G1T38 (100 mg/kg), osimertinib (10 mg/kg), or the combination by oral gavage for 28 days (n=10).

(C) HCC827/ER1, a human NSCLC that harbors EGFR exon 19 del E746-A750, has acquired resistance to erlotinib (and other EGFR TKIs) through an approximately 5- to 10-fold amplification of the MET gene. To evaluate the combination of G1T38 and osimertinib, tumor-bearing mice were given once daily doses of G1T38 (100 mg/kg), osimertinib (5 mg/kg), or the combination by oral gavage for 25 days (Crown Bioscience, Beijing, China, n=10).

TABLE 1. TIME TO TUMOR DOUBLING

NSCLC tumor model	Treatment	Median (days)
EGFR$^{L858R/T790M}$	vehicle	6
	afatinib	13.5
	afatinib + G1T38 (50)	22
	afatinib + G1T38 (100)	27
	erlotinib	6
	erlotinib + G1T38 (50)	7
	erlotinib + G1T38 (100)	18
EGFR^{ex20ins}	vehicle	6
	osimertinib	7.5
	osimertinib + G1T38	14
EGFR^{ex19del}/MET+	vehicle	6
	osimertinib	13
	osimertinib + G1T38	>25

Median time to tumor doubling was calculated from data shown in Figure 5.

SUMMARY

G1T38 treatment demonstrated single agent activity in preclinical *in vitro* and *in vivo* models of NSCLC. Additionally, combination therapy with agents targeting NSCLC driver mutations significantly enhanced efficacy.

- In a panel of NSCLC PDX models, there was a range of G1T38-associated efficacy with certain genetic alterations (KRAS and EGFR) demonstrating increased sensitivity, while others (RB1) demonstrated resistance.
- In a screen of lung cancer cell lines, the addition of G1T38 to targeted treatments demonstrated the most synergy when combined with inhibitors of EGFR, ALK, and HER2.
- Combination treatment in a variety of *in vivo* tumor models (KRAS^{mut}, ALK fusion, and EGFR^{mut}) further validates that the addition of G1T38 enhances the efficacy of other targeted therapies.

Preliminary *in vitro* signaling data suggests the enhanced efficacy of combination treatment may be due to more profound suppression of RB phosphorylation coupled with an enhancement of a pro-apoptotic phenotype when compared to either single agent treatment.

The addition of G1T38 was able to overcome both primary and acquired resistance to single agent EGFRi therapy in EGFR^{mut} NSCLC models:

- G1T38 + erlotinib reversed resistance to single-agent erlotinib treatment in an EGFR$^{L858R/T790M}$ tumor model; the T790M mutation renders erlotinib ineffective.
- The addition of G1T38 to afatinib treatment almost doubled the time to afatinib resistance in an EGFR$^{L858R/T790M}$ tumor model.
- EGFR$^{L858R/T790M}$ tumors progressing on single-agent afatinib showed stabilization of tumor growth by adding G1T38 to afatinib treatment.
- In an osimertinib resistant EGFR^{ex20ins} tumor model, G1T38 + osimertinib significantly slowed tumor growth; NSCLCs with EGFR^{ex20ins} mutations are generally insensitive to EGFR inhibitors in the clinic.
- The addition of G1T38 to osimertinib treatment significantly enhanced efficacy in an EGFR^{ex19del}/MET+ tumor model; MET amplification is a known mechanism of acquired resistance to EGFR inhibitors.

A Phase 1b/2 trial of G1T38 + osimertinib is currently enrolling patients with EGFR-mutant NSCLC (trial G1T38-03, NCT03455829).