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Pre-clinical evaluation of a potent and orally bioavailable next-generation inhibitor targeting the family of mutants that drive oncogenic BRAF dimer formation

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ABSTRACT

BACKGROUND
The canonical BRAF V600E (Class I) mutation is a potent oncogene which is uniquely active as a RAS-independent monomer, and which has been successfully targeted by several FDA-approved inhibitors. While active against monomeric BRAF V600E, these first generation BRAF inhibitors induce paradoxical activation of RAS-driven BRAF dimers in cells expressing wild-type RAF, and this can lead to secondary malignancies[1-4]. More recently, numerous non-canonical BRAF mutations including BRAF-fusions have been described as oncogenes that drive RAS-independent (Class II) or RAS-dependent (Class III) dimers. These non-canonical mutant BRAF dimers are resistant to the first-generation drugs. Discovery of an inhibitor directed against the family of dimeric BRAF mutations which avoids paradoxical activation therefore presents a major unmet need.

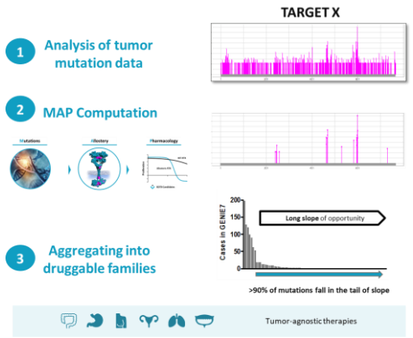
METHODS
We applied our proprietary Mutation-Allostery-Pharmacology (MAP) platform technology to identify and validate a group of previously unrecognized and un-drugged oncogenic mutations that may have important therapeutic implications. We further demonstrate that this ensemble of oncogenic BRAF mutants can form the basis of a differentiated drug discovery program aimed at identifying small molecule MasterKeys that potently and selectively target these families of dimeric BRAF mutations.

RESULTS
Herein, we describe a small molecule inhibitor with potent anti-proliferative activity directed against tumor cells harboring dimeric BRAF mutations and which are devoid of paradoxical RAF activation. A leading exemplar of BDTX compound is an orally available inhibitor that achieve target engagement of mutant BRAF dimer in vivo and robust anti-tumor efficacy in mutant BRAF monomer and dimer mouse models.

CONCLUSION
These data support continued development of rationally designed molecules targeting a broad range of non-canonical BRAF dimer-promoting mutations to extend the prospect of precision medicine for cancer patients with BRAF mutations.

Tumor-Agnostic Precision Oncology Medicine Strategy

Black Diamond Therapeutics (BDTX) utilizes its proprietary technology platform, Mutation–Allostery-Pharmacology (MAP), to reveal and validate previously unrecognized and undrugged oncogenic mutations from tumor mutation data with important therapeutic implications. These mutations are then aggregated into clusters for development of a mutation-spectrum selective MasterKey inhibitor.

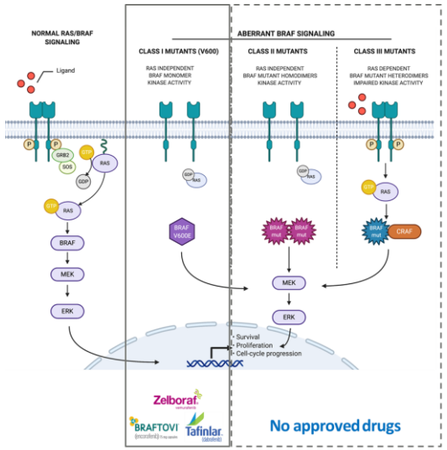


Opportunity to Address Multiple Gaps for Approved Agents

The non-canonical BRAF Class II and III mutants include point mutations, fusions, and deletions which lead to aberrant cell survival and proliferation.

FDA-approved BRAF V600E-selective inhibitors, such as encorafenib and vemurafenib, are inactive against mutant BRAF dimers and show limited clinical effectiveness in non-V600 mutant BRAF-dependent tumors. Furthermore, these drugs induce paradoxical activation leading to secondary malignancies[1-4].

Our goal is to develop an orally available, selective, and potent inhibitor of mutant BRAF dimers with no paradoxical activation, for the treatment of patients with solid tumors harboring dimeric BRAF mutations.



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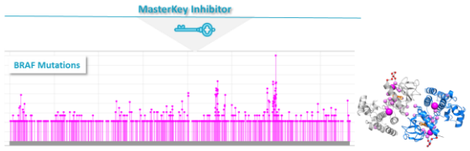
Development of Master Key Inhibitor Against Dimeric BRAF Mutations

The MAP platform was applied to identify and validate a group of previously unrecognized and un-drugged dimeric BRAF mutation clusters. To date, 28 non-canonical BRAF mutations including previously unaddressed have been validated to be oncogenic. A MasterKey inhibitor, BDTX-A, against aggregated mutant BRAF dimers has been discovered.



MasterKey Inhibitor Against Mutant BRAF Dimers

BDTX-A potently inhibits the proliferation of Ba/F3 transformants expressing a spectrum of non-canonical Class II/III mutations. In contrast, encorafenib has lower activity against these mutant BRAF dimers. This suggests that BDTX-A has potent activity against a broad range of non-canonical BRAF dimer-promoting mutations.

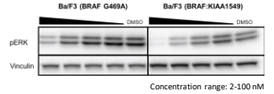
Color coding BDTX-A potency vs Encorafenib (IC_{50})	Lower	Equivalent (± 3X)	Higher

Mutation	Class Mutation	BDTX-A
F247L	III	
G464V	II	
G464R	II	
L597R	II	
L597Q	II	
G469R	III	
G469A	II	
G466A	II	
L597V	II	
K601N	II	
K601E	II	
Deletion AA487-492	II	
KIAA fusion	II	
SND-1 fusion	II	

Cell proliferation Assay

Demonstration of On-Target Activity

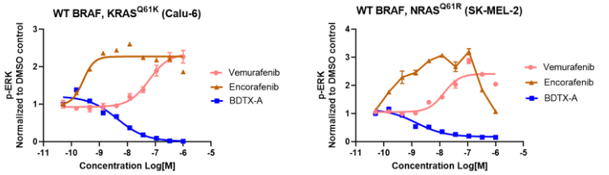
BDTX-A shows dose-dependent inhibition of pERK, which is downstream of BRAF, in mutant BRAF dimer expressing Ba/F3 cells.



Concentration range: 2-100 nM

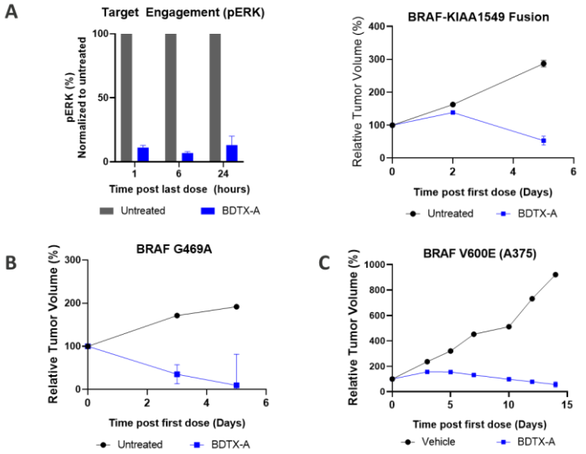
Avoidance of Paradoxical Activation

In contrast to approved drugs that are known to induce paradoxical activation, BDTX-A treatment of cells harboring WT BRAF does not lead to an increase in pERK.



In vivo Efficacy in Multiple BRAF Mutant Mouse Models

BDTX-A demonstrates robust anti-tumor activities in mutant BRAF dimer-driven allograft models (Ba/F3 expressing BRAF-KIAA1549 or BRAF G469A) and BRAF V600E-driven xenograft model (A375). The animals treated with BDTX-A show sustained and lower pERK level in tumors compared to untreated animals (BRAF-KIAA1549 fusion data shown).



Dose schedules for mutant BRAF dimer (A) BRAF-KIAA1549 fusion and (B) BRAF G469A studies were QD for 5 days at 300 mg/kg po. Tumor samples were collected at the indicated time after the last dose.

Dose schedule for (C) BRAF V600E (A375) was QD for 14 days at 100 mg/kg po.

SUMMARY

Dimeric BRAF mutations result in aberrantly active BRAF which leads to activation of its downstream signaling effectors, MEK and ERK. These non-canonical BRAF mutations are found in different types of cancer and may be oncogenic drivers. However, there are no approved drugs targeting these mutant BRAF dimers.

- **We discovered a small molecule inhibitor, BDTX-A, that is potent against a cluster of dimeric BRAF mutations.**
- **BDTX-A inhibits the proliferation of cells driven by these dimeric BRAF mutations without inducing paradoxical activation.**
- **BDTX-A demonstrates in vivo efficacy by promoting regression of tumors expressing mutant BRAF monomer or dimer.**

These data warrant continued development of BDTX-A for the treatment of patients expressing BRAF oncogenes in a tumor agnostic manner.

REFERENCES
[1] *Belum et al., 2015;* [2] *Su et al., 2012;* [3] *Hatzivassiliou et al., 2010;* [4] *Poulikakos et al, 2010.*

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