# ASN003, a highly selective inhibitor of B-RAF and PI3 kinases, shows strong antitumor activity in a B-RAF inhibitor resistant patient-derived xenograft model

158

Scott K. Thompson[1], Roger A. Smith[1], Niranjan Rao[1], Michael J. Wick[2], Sanjeeva P. Reddy[1]

[1]Asana BioSciences, 1009 Lenox Drive, Suite 106, Princeton Pike Corporate Center, Lawrenceville, NJ 08648, USA, and [2]South Texas Accelerated Research Therapeutics, 4383 Medical Drive, San Antonio, TX 78229

## Abstract

The RAS-RAF-MEK and PI3K-AKT-mTOR pathways are two major signaling pathways involved in human cancer. Components of these two pathways are frequently mutated in a wide variety of solid tumors. Concurrent double mutations in the two pathways are also observed quite often in a broad range of tumor types. Additionally, inhibition of one of these pathways often leads to the upregulation of the other pathway and development of resistance. In preclinical models, combined inhibition of both pathways has been shown to impart greater efficacy as compared to inhibition of either pathway alone. ASN003 is a novel, highly selective, small-molecule inhibitor of both RAS-RAF and PI3K pathways, discovered using a rational design approach. ASN003 shows potent inhibitory activity against B-RAF and PI3K kinases (low nM $IC_{50}$). Within the PI3K family, ASN003 has high selectivity for inhibition of PI3Kα and PI3Kδ over PI3Kβ. In a panel of 292 kinases, ASN003 showed high selectivity for inhibiting B-RAF and PI3 kinases, and associated mutant kinases. In cell-based mechanistic studies, ASN003 inhibited phosphorylation of ERK, AKT and S6, and showed strong antiproliferative activity ($IC_{50}$ = 60-300 nM) in cell lines with B-RAF and PI3K pathway mutations as well as in vemurafenib-resistant cell lines. In pharmacodynamic studies in multiple tumor models, ASN003 showed strong inhibition of the phosphorylation of downstream targets of B-RAF and PI3K, confirming appropriate target engagement. In *in vivo* efficacy studies, ASN003 showed strong tumor growth inhibition or regression in multiple tumor xenograft models, including A375 (B-RAF V600E mutation), RKO (B-RAF V600E and PIK3CA mutations), and A2058 (B-RAF V600E mutation and PTEN loss). We now report that ASN003 also showed strong tumor growth inhibition (>80%) in a patient-derived xenograft (PDX) model established from a relapsed patient with progressive B-RAF mutant melanoma who showed initial response to vemurafenib. Sequencing analysis showed that the vemurafenib resistant tumor acquired a concurrent PIK3CA mutation. Dual targeting of the B-RAF and PI3K pathways with ASN003 has the potential to treat and/or prevent the acquired resistance to selective B-RAF inhibitors, and may also treat a broader patient population and provide greater efficacy and survival benefit than selective B-RAF inhibitors or selective PI3K pathway inhibitors alone. ASN003 is currently in Phase I clinical development in patients with advanced solid tumors, including tumors with B-RAF V600 mutation or PI3 kinase pathway alterations or PTEN loss.

## *In Vitro* Pharmacology – Biochemical

| | Assay | Vemurafenib $IC_{50}$ (nM) | ASN003 $IC_{50}$ (nM) |
|---|---|---|---|
| Biochemical Assays | B-RAF$^{V600E}$ | 17 | 1 |
| | PI3Kα | >10,000 | 16 |
| | mTOR | >10,000 | 160 |

The $IC_{50}$ values for inhibition of the kinases by ASN003 and vemurafenib were determined in a biochemical assay using purified proteins of B-RAF$^{V600E}$, PI3Kα and mTOR enzymes. ASN003 showed potent inhibition of all three kinases whereas vemurafenib inhibited only B-RAF$^{V600E}$

## *In Vitro* Pharmacology – Cell-based

| | Assay | Vemurafenib $IC_{50}$ (nM) | ASN003 $IC_{50}$ (nM) |
|---|---|---|---|
| A375 Cells (B-RAF mutant, melanoma) | pERK | 54 | 68 |
| | Proliferation | 170 | 140 |
| RKO Cells (B-RAF / PI3K double mutant, colorectal) | pERK | 150 | 14 |
| | pS6RP | NT | 220 |
| | pAKT (T308) | NT | 180 |
| | Proliferation | >10,000 | 290 |

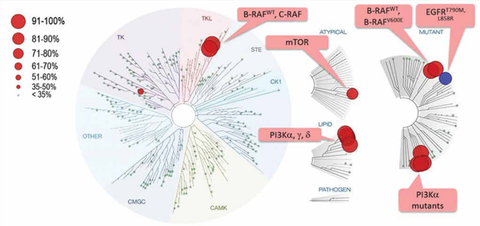
Inhibition of phosphorylation of ERK, S6RP and AKT$^{T308}$ was determined by in-cell Western method after cells were treated for 3 h with ASN003 or vemurafenib. Antiproliferative activity was determined by XTT method after 72 h treatment of the cells with compounds.

## PI3K Isozyme Selectivity of ASN003

| Assay | ASN003 $IC_{50}$ (nM) | Pictilisib (GDC-0941) $IC_{50}$ (nM) |
|---|---|---|
| B-RAF$^{V600E}$ | 1 | >10,000 |
| PI3Kα | 16 | 3 |
| PI3Kβ | 690 | 33 |
| PI3Kγ | 97 | 75 |
| PI3Kδ | 6 | 3 |
| mTOR | 160 | 580 |

The isozyme selectivity of ASN003 and pictilisib were determined in a biochemical assay using purified proteins of PI3K isoforms. ASN003 showed >40 fold selectivity for PI3Kα compared to the PI3Kβ isozyme.

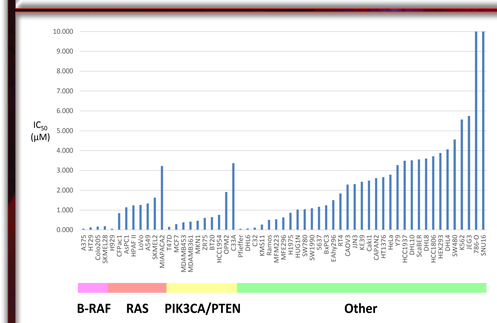
## Kinase Selectivity of ASN003




ASN003 showed very high selectivity for B-RAF and PI3 kinases in a screen of 292 kinases in biochemical assay format at a ASN003 concentration of 1 µM.

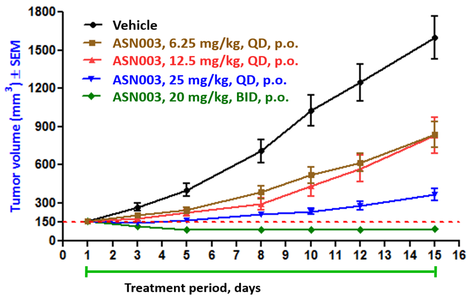
Image generated using TREEspot™ Software Tool and reprinted with permission from KINOMEscan®, a division of DiscoveRx Corporation, ©DISCOVERX CORPORATION 2010.

## Antiproliferative Activity in a Broad Panel of Cell Lines



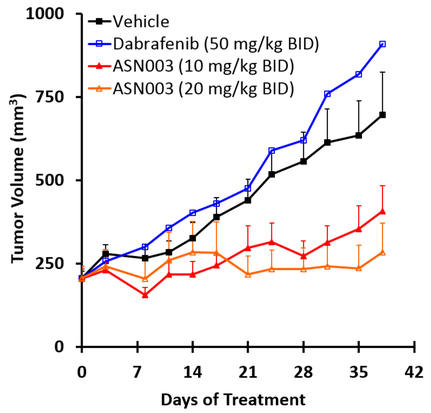

In a panel of 57 cells lines, ASN003 showed a preference for potent inhibition of proliferation of cell lines harboring B-RAF and/or PI3K pathway mutations.

## Tumor Growth Inhibition (A375 Model)



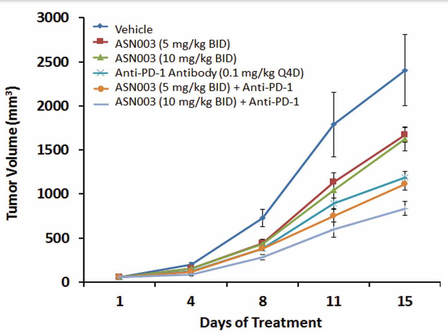

ASN003 showed strong tumor growth inhibition in the A375 melanoma tumor mouse xenograft model driven by the B-RAF$^{V600E}$ mutation. Tumor regression was observed at the 20 mg/kg BID PO dose.

## Tumor Growth Inhibition in PDX Model




The ST052C tumor is derived from a patient who had acquired resistance to vemurafenib. The tumor harbors the B-RAF$^{V600E}$ mutation and an acquired PIK3CA mutation.

## Tumor Growth Inhibition in Combination with Anti-PD-1 Antibody (B16F10 Model)




ASN003 showed additive tumor growth inhibition when dosed at 10 mg/kg BID in combination with anti-PD-1 antibody at 0.1 mg/kg Q4D in a B16F10 murine melanoma model.

## ASN003 Shows Anti-proliferative Activity in B-RAF Inhibitor Resistant Cells

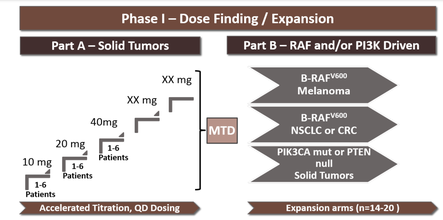
| Compound | Vemurafenib-Sensitive A375, $IC_{50}$ (nM) | Vemurafenib-Resistant A375, $IC_{50}$ (nM) | Ratio |
|---|---|---|---|
| Vemurafenib | 0.17 | 8.2 | 48 |
| **ASN003** | **0.16** | **0.64** | **4** |

Vemurafenib (B-RAF inhibitor) resistant clones were generated by exposing A375 cells to increasing concentrations of vemurafenib for several weeks. The data for one of the resistant clones is shown in the table.

## Preclinical ADME/Safety Profile

- Good oral bioavailability in rat (100%) and dog (38%)
- Not a Pgp substrate
- Low risk for drug-drug interactions: No significant inhibition of major CYP isoforms ($IC_{50}$ > 5 µM), or induction of CYP3A4 and 1A2
- No significant hERG channel blockade ($IC_{50}$ > 2.6 µM)
- Negative in mutagenicity studies (AMES and chromosomal aberration)
- No significant findings on CV, CNS and Respiratory systems in safety pharmacology studies

## A Phase I, Open-label, Dose-finding and Cohort Expansion Study of ASN003 in Subjects With Advanced Solid Tumors




- First three cohorts have completed DLT phase
- Good plasma exposure achieved with PK profile consistent with QD dosing
- ASN003 is considered well-tolerated, to date only mild (CTC G1) drug-related adverse events reported

**Exploratory biomarkers:** pERK, pAKT$^{S473}$, Cyclin D, Ki-67

## Summary

- **First-in-Class dual B-RAF and PI3K inhibitor**
- ASN003 has potent activity against B-RAF, and PI3Kα and δ kinases (PI3Kβ-sparing)
- High target selectivity in kinome screening
- Robust antiproliferative activity and *in vivo* efficacy in B-RAF inhibitor resistant models including a patient-derived xenograft model harboring B-RAF$^{V600E}$ mutation and an acquired PIK3CA mutation
- Additivity of *in vivo* efficacy in combination with anti-PD-1 antibody in a murine melanoma model
- Phase I clinical study is in progress; well tolerated at the doses tested to date
- **ASN003** is in clinical development for the potential treatment of patients with B-RAF or PI3K-driven cancers, including patients pretreated with current B-RAF inhibitors. ASN003 may be developed as monotherapy or in combination with checkpoint inhibitors or standard of care.

**References**

1. J. A. McCubrey et al, *Oncotarget* **2012**, 3 (10): 1069-1111
2. J.A. Engelman et al, *Nat. Med* . **2008**, 14 (12): 1351-1356

ASANA BIOSCIENCES

AACR Annual Meeting • Washington, DC • April 1-5, 2017

asanabio.com