# Potential Small-Molecule Inhibitors of the Tiam1 Oncogene Product

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## Abstract

**The T-cell lymphoma Invasion and Metastasis-inducing factor (Tiam)1 is an established proto-oncogene that drives cancer cell migration and metastasis in multiple cancer settings. The strong correlation between Tiam1 integrity, tumor grade, and patient survival render it an important diagnostic and prognostic factor in multiple human malignancies. Tiam1 is a guanine nucleotide exchange factor (GEF) that facilitates the activation of the small GTPase Rac1, thereby controlling cytoskeletal organization, cell polarity, motility, and invasion. In light of the clinical relevance of Tiam1 signaling, we investigated the possible utility of small-molecule inhibitors that target Tiam1 as a therapeutic intervention strategy in relevant tumor models. We used an in silico computational approach to screen seven million commercially available compounds for candidate drugs that bind to a specific patch on Tiam1's surface. Of these, we identified 13 compounds that inhibit the migration of Tiam1-expressing cells without affecting overall viability. Our studies open the door for a new targeted therapy approach in Tiam1-relevant cancers.**

## Introduction

- > 20% of cancer patients develop metastatic disease, which is a crucial determinant of disease severity and outcome.
- Tiam1 is a proto-oncogene that facilitates cell metastasis and invasion in multiple malignancies, including colorectal, lung, ovarian, and breast cancers.
- Tiam1 is a Dbl-family GEF that stimulates signaling from the small GTP-binding protein Rac1.
- Activation of the GTPase Rac1 by Tiam1 stimulates reorganization of the cytoskeleton, cell cycle progression, and cell polarity.
- We identified 1055Tyr as a tyrosine-phosphorylation site in Tiam1 that modulates its GEF activity.
- 1055Tyr resides within a novel putative phosphorylation consensus sequence conserved amongst multiple GEFs from the Dbl family.
- These findings offer the possibility of inhibiting Tiam1 with small-molecule inhibitors that target the phosphorylation site.
- The AtomNet® model uses a single global convolutional neural net trained to predict Ki and IC50 values from several million small molecule affinity labels and several thousand protein structures.
- Using this model, we have screened 7M commercially available compounds and identified 82 potential small molecule inhibitors of Tiam1 that are predicted to bind to the surface surrounding 1055Tyr.
- Of these, we honed in on 13 compounds that inhibit the migration of Tiam1-expressing cancer cells without inducing cytotoxicity.
- Ongoing studies will further narrow the list to those inhibitors that function in a Tiam1-specific manner.

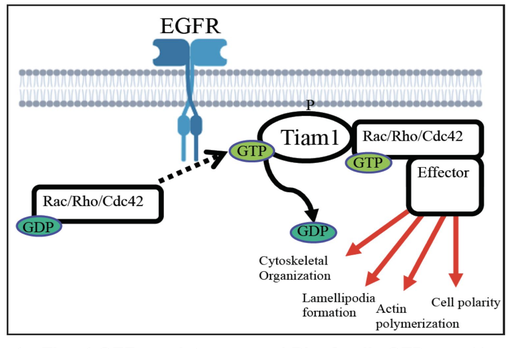


***Figure 1—Tiam1 GEF regulates several Rho family GTPases***: Upon epidermal growth factor binding, EGFR facilitates Tiam1 phosphorylation via downstream tyrosine kinase activity. Phosphorylated Tiam1 facilitates GTPase conversion from the GDP-bound, inactivate state, to the GTP-bound active state. Rho family GTPases interact with several effector proteins which mediate cellular activities related to motility, chemokinesis, implicated in cancer metastasis

## Results

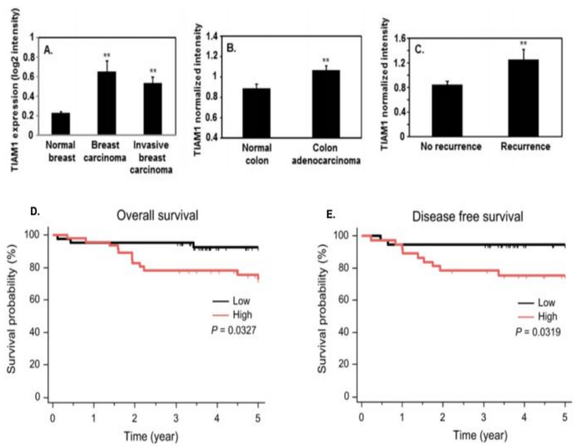


***Figure 2 —Tiam1 expression level is associated with cancer:*** *(A) Tiam1 expression is higher in breast carcinoma (B) colon adenocarcinoma (C) and rate of cancer recurrence. (D) Kaplan-Meier curve of the overall survival with high or low expression of Tiam1, and (E) percent disease free survival with high or low expression of Tiam1. (Adapted from: Goel et al. Nature 2019)*

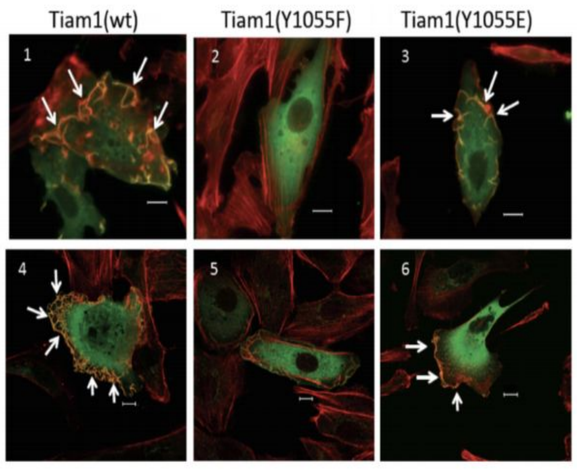


**Figure 3 —** *MDA-MB-231 cells were transfected with the indicated constructs and immunofluorescence microscopy utilized to visualized the cellular distribution of Tiam1 (green) and the actin cytoskeleton (red). Co- localization is yellow, and lamellipodia are indicated by arrows. Scale bar = 10 µm.*

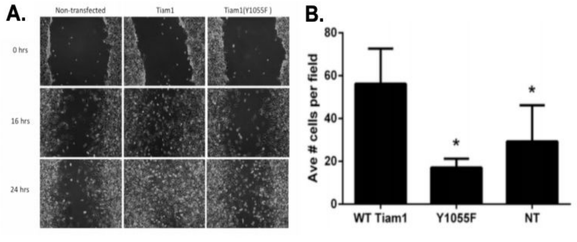


***Figure 4 —Wound Healing Assay with Tyrosine Mutation:*** *(A, B) NIH 3T3 cells transfected to overexpress TIAM1 were scratched using a pipette tip and wound closure was measured using a microscope at the indicated time points. A mutant form of NIH 3T3, in which the tyrosine residue was replaced with phenylalanine (Y1055F), was scratched in the same manner and compared. The replacement of tyrosine (potential phosphorylation site) with an incomparable amino acid residue significantly reduces the rate of wound closure.*

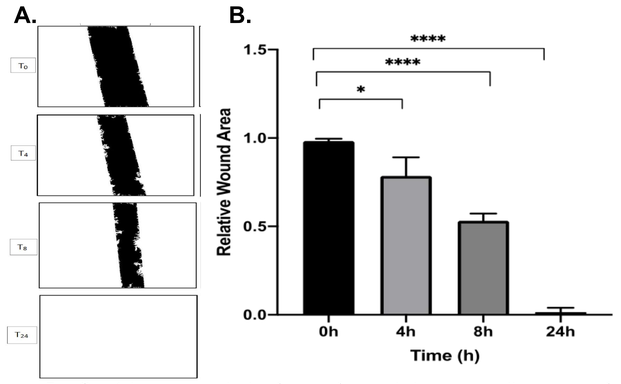


**Figure 5 —***Scratch assay analysis of area of wound closure over the course of 24h. Panel A demonstrates the 'wound' area as calculated by Fiji software. Panel B represents the quantification of the areas of wounds at each time point normalized to 0h. Data is representative of three experiments. Asterisks denote statistical significance of < 0.05.*

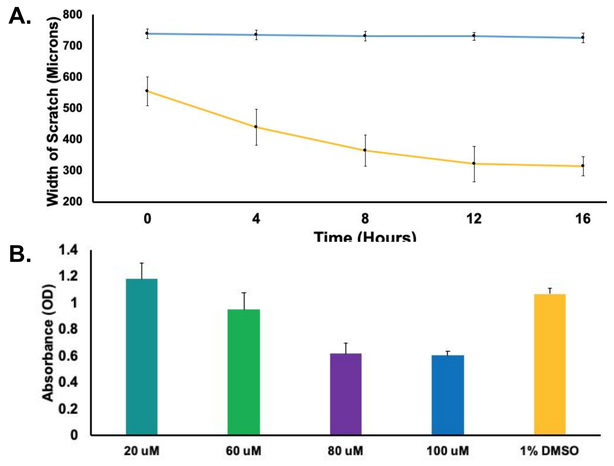


***Figure 6 — Wound closure of MDA-MB-231 cells with compound treatment.*** Panel A: wound closure over time as quantified by incucyte imaging software. Conditions: Negative 1% DMSO control (yellow), effective compound 320880342 at 100 µM concentration (blue). Panel B: MTT assay, measured in optical density (OD), with varied concentrations of 320880342 to determine (1) whether the compound created cytotoxic conditions (resulting in mostly dead cells, unable to reduce the MTT) and (2) whether the compound is effective at lower concentrations (dose responsiveness).

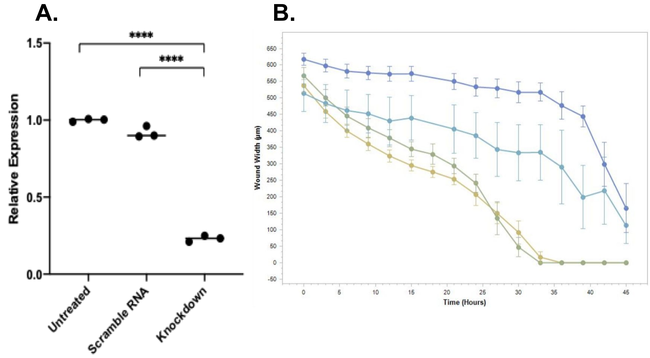


***Figure 7 — Wound closure of A549 cells with compound treatment and Tiam1 silencing.*** *Panel A displays Tiam1 expression levels following 24h of siRNA knockdown relative to untreated control as well as to scrambled siRNA. Gene expression was measured with RT-qPCR and normalized to beta actin. Asterisks denote statistical significance of < 0.05. Panel B quantifies imaging of 'wound' closure in A549 cells over the course of 48h following transfection with Tiam1 siRNA or scramble RNA control. Conditions: treated with compound without Tiam1 siRNA(blue), treated with compound and Tiam1 siRNA (teal), no compound with DMSO with Tiam1 siRNA (yellow), and no compound without siRNA (green).*

## Conclusions

Aberrant expression of Tiam1 in multiple malignancies is associated with poor prognosis and greater rate of recurrence, underscoring the potential utility of this protein as an intervention target in cancer. We have discovered that Substitution of Tyr1055 in Tiam1 to phosphor-mimetic (Glu) and phospho-defective (Phe) residues impact cell motility in a manner consistent with an activation scenario that involves reversible phosphorylation of 1055Tyr as a regulatory conformational switch.

Ongoing studies employ high-throughput cell motility inhibitor screens and in vitro binding assays to ascertain selectivity of the compounds toward Tiam1 and to initiate medicinal chemistry studies that will optimize affinity and efficacy.

## Future Directions

- Studies in A549 (lung cancer) cells will be complemented by work in colorectal (SW480) and breast (MD-MB-231) cancer cells that express Tiam1.
- Fluorescence resonance energy transfer (FRET), microcalorimetry and anisotropy binding assays will be utilized to quantify the binding between candidate inhibitors and Tiam1.
- Promising candidate Tiam1 inhibitors will be examined in an in vivo tumorigenesis and metastasis model using Tiam1-expressing xenograft mouse models.

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