# Development of angiopoietin-1 receptor (TIE2) agonist for retinal vascular disorders

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## Introduction

Retinal vascular disorders including diabetic retinopathy, and age related macular degeneration are leading causes of blindness globally. While these various retinal vascular diseases have distinct causes, they share a common pathophysiology, including uncontrolled neovascularization that results in leaky and bleeding blood vessel.

Current treatments have focused on agents that inhibit vascular endothelial growth factor (VEGF), which is elevated in people with retinal vascular disorders and plays a major role in angiogenesis and vascular permeability. Unfortunately, not all patients respond to anti-VEGF drugs. Such suboptimal outcomes are largely because VEGF is only one component of a complex pathway causing retinal vascular disorders.

Recent studies have found a role of activated angiopoietin-1 receptor (TIE2) — a tyrosine kinase that promotes vascular stability — in preventing retinal vascular disorders. To discover molecules that activate TIE2 and restore vascular stability, we screened a phage display library and identify TC1, a cyclic peptide that binds to TIE2 and stimulate its phosphorylation.

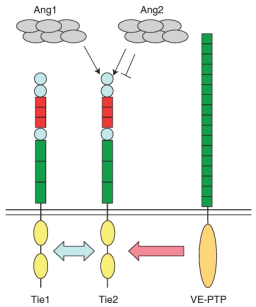


**Figure 1**: Components of the angiopoietin–TIE2 pathway. Ang1 and Ang2 are multimeric ligands of TIE2, and while Ang1 is an agonist, Ang2 can be agonist or antagonist depending on the context. Ang: Angiopoietin; VE PTP: vascular endothelial protein tyrosine phosphatase.
Image from Durston and Daly, 2012; Cold Spring Harb Perspect Med

## Methods

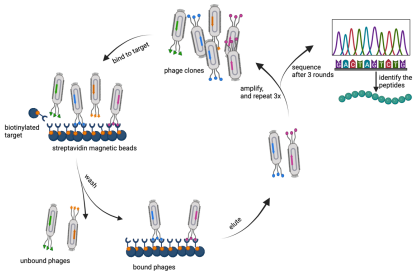


**Figure 2**: We used phage display library screen to identify TC1. Approximately one billion phage clones containing unique cyclic peptides were incubated with biotinylated TIE2. After several washing, bound phages were eluted, and amplified. This was repeated 3x and the eluted phages were sequenced to determined the bound peptides.

## Results

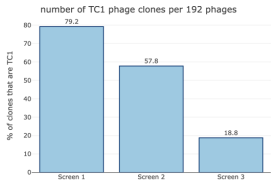


**Figure 3**: Distribution of TC1 clones after three successive screening.

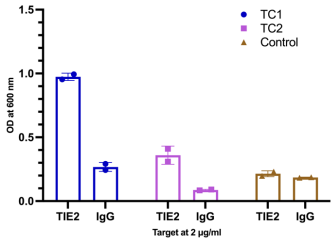


**Figure 4**: Confirmation of TC1 affinity to TIE2 with phage enzyme-linked immunosorbent assay. This data shows that the affinity of TC1 clone to TIE2 is stronger than to IgG. TC2 is another phage clone that showed affinity to TIE2; Control is a random phage clone

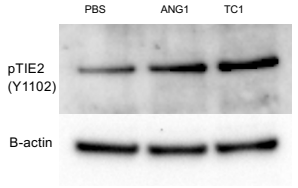


**Figure 5**: TC1 stimulates TIE2 phosphorylation in HUVEC cell line. This data shows that TC1 binds to TIE2 and promotes TIE2 phosphorylation since pTIE2 level is higher in TC1 treated cells than the baseline (PBS) control. PBS and ANG1 are baseline and positive controls respectively. pTIE2: phospho-TIE2.

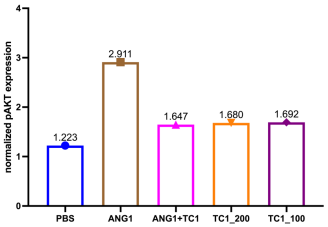


**Figure 6**: TIE2 phosphorylation was evaluated by pAKT level in HUVEC cell line (activation of TIE2 (pTIE2) leads to downstream activation of AKT (pAKT)). This data shows that TC1 is a competitive agonist with ANG1, because adding TC1 with ANG1 removes ANG1 agonist activity. TC1_200: TC1 at 440 µg/ml; TC1_220: 220 µg/ml

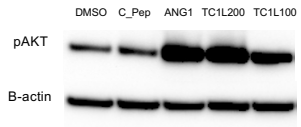


**Figure 7**: Linear TC1 also stimulate pAKT in HUVEC. C_Pep: random peptide; TC1L200: linear TC1 at 440 µg/ml; TC1L100: linear TC1 at 220 µg/ml; DMSO was used as baseline here because the linear TC1 is water insoluble

## Conclusions

We show that TC1 has a strong affinity to TIE2, and stimulates its phosphorylation and downstream activation of pAKT. TC1 is a potential drug candidate for retinal vascular disorders.