THE TIE2 PATHWAY: What Retina Specialists Need to Know

Report of a Roundtable Discussion held July 13, 2017

Over the past few decades, understanding the role of vascular endothelial growth factor (VEGF)—including the development of anti-VEGF agents—has revolutionized treatment of diseases such as age-related macular degeneration (AMD) and diabetic macular edema (DME). But in spite of the benefits achieved with VEGF suppression, many patients have a suboptimal response, underscoring the need to identify and address other therapeutic targets.[1] Emerging basic science and clinical research are revealing a different pathway and set of therapeutic targets: the tyrosine kinase receptor, Tie2, and its negative regulators, including vascular endothelial-protein tyrosine phosphatase (VE-PTP) and angiopoietin-2 (Ang-2).

In this expert roundtable discussion, we will review what is known about the Tie2 pathway, its significance in retinal vasculopathies, and the therapeutic approaches currently under investigation to manipulate its activity. We will differentiate and discuss the status of clinical research into these approaches, and finally, contextualize our expectations for how they will impact clinical practice.

FUNCTIONS OF THE TIE2 PATHWAY

PRAVIN U. DUGEL, MD **This pathway is complex, so let's begin with the basics. Dr. Campochiaro, can you please outline the Tie2 pathway and its importance in the body?**

PETER A. CAMPOCHIARO, MD Tie2 is a tyrosine kinase receptor expressed primarily in the vascular endothelium, and it plays a very important role in vascular development. Without either Tie2 or its binding partners, angiopoietin-1 or angiopoietin-2 (Ang-1 and Ang-2, respectively), vascular development is abnormal.[2]

This pathway is critical for the development of normal blood vessels, and in adults, it is important for vascular stabilization. Tie2 is constitutively activated by Ang-1 in endothelial cells throughout the body, resulting in a cascade of events that help maintain a stable, quiescent vasculature.[2] In the retina, activated Tie2 controls endothelial cell proliferation, barrier function, and intercellular contacts, stabilizing vessels and the blood-retinal barrier.[3] Tie2 is inactivated in certain disease processes, which allows for vascular leakage and pathologic angiogenesis.[2]

DUGEL **Can you describe what is meant by "vascular stabilization" in more detail?**

CAMPOCHIARO A stable vasculature is one in which vessels are operating in a state of homeostasis. Pericytes, endothelial cell junctions, and the extracellular matrix are intact. A stable vasculature, particularly in the retina, is non-leaking: it does not allow plasma or bone marrow-derived cells into the tissue, nor does it respond to transient fluctuations in stimulating factors such as VEGF.

> Ang-1 is the endogenous agonist for Tie2: when Ang-1 binds and stimulates phosphorylation of Tie2, it initiates a cascade of signaling pathways that ultimately lead to vascular stability.

Indeed, as it pertains to this pathway, in order for the vascular endothelium to be stimulated by VEGF, Tie2 has to be downregulated.[2-4] So in a sense, Tie2 acts as a sort of rheostat to control how much the endothelial cells will respond to other stimulating factors.[4] VEGF is thought to be the primary stimulus for both vascular leakage and pathologic angiogenesis; when activated, the Tie2 pathway counters that by reducing the endothelial cell response to VEGF.[4,5]

DUGEL **How is the Tie2 pathway regulated?**

PETER K. KAISER, MD Unlike the VEGF pathway, there are a couple of regulators of Tie2. The first class of regulators are the angiopoietins, Ang-1 and Ang-2. Ang-1 is the endogenous agonist for Tie2: when Ang-1 binds and stimulates phosphorylation of Tie2, it initiates a cascade of signaling pathways that ultimately lead to vascular stability.[2,5]

We can think of Ang-2 as an antagonist, though it is actually a partial agonist.[6] Ang-2 binds to Tie2, but does not lead to its phosphorylation. Thus, in most cases, Ang-2 works by blocking Ang-1 and

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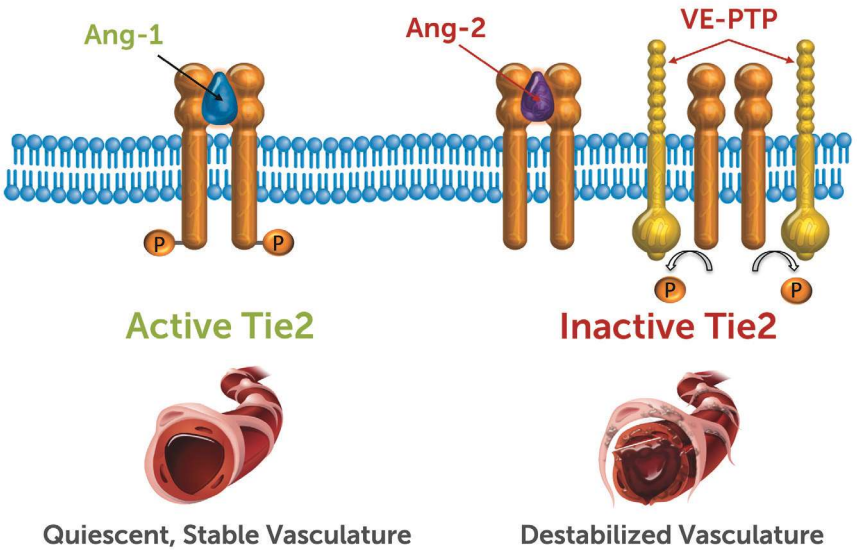

FIGURE 1 Schematic of the Tie2 pathway. Ang-1 binds Tie2, causing its phosphorylation and a consequent cascade of cellular events that leads to vascular stabilization. Tie2 activation can be prevented via dephosphorylation by VE-PTP or competitive binding by Ang-2, either of which leads to vascular destabilization.

preventing Tie2 activation.[1] This, in turn, leads to destabilization of the vasculature and enhanced responsiveness to inflammatory mediators, including VEGF.[5] Put simply: Ang-1 causes Tie2 activation, and Ang-2 inhibits Tie2 activation.

Tie2 can also be deactivated via a separate mechanism: an intracellular enzyme called vascular endothelial-protein tyrosine phosphatase (VE-PTP), which directly dephosphorylates Tie2 (Figure 1).[5,7] VE-PTP also associates with VE-cadherin, which itself plays a key role in maintaining vascular endothelial junctional stability; but VE-PTP has been shown to deactivate Tie2 and destabilize vascular endothelial cells.[7]

DUGEL You are describing two different ways to regulate Tie2. One appears to be between Ang-1 and Ang-2, and the other with VE-PTP. Could we differentiate these more clearly? Is one of these strategies better than the other in terms of a potential drug target?

KAISER In hypoxia or hyperglycemia, both Ang-2 and VE-PTP are upregulated.[1,2,4] Both can cause Tie2 to be dephosphorylated, or inactivated. Blocking either one, then, should cause Tie2 activation. However, in order for Ang-2 inhibition to succeed in activating Tie2, Ang-1 is necessary to actually bind and cause phosphorylation of the Tie2 receptor.[5,8] In the absence of Ang-1, Ang-2 inhibition would not cause Tie2 activation.

In contrast, blocking VE-PTP leads to Tie2 phosphorylation irrespective of the level of Ang-1. This is a very important point, because in conditions like diabetes or AMD, where there are elevated levels of Ang-2, there tend to be low levels of Ang-1 in the extracellular space.[1] So even if Ang-2 is blocked, if Ang-1 is not available to activate Tie2, we may not get the vascular stabilization we want. But by blocking VE-PTP, we get Tie2 receptor activation, regardless of the levels of Ang-1 or Ang-2.

> VEGF is thought to be the primary stimulus for both vascular leakage and angiogenesis; when activated, the Tie2 pathway counters that by reducing the endothelial cell response to VEGF.

CAMPOCHIARO By inhibiting VE-PTP, Tie2 is not only activated, but also more responsive to both Ang-1 and Ang-2—essentially converting Ang-2 to an agonist. Since there are high levels of Ang-2 in ischemic tissue, this serves to amplify the Tie2-activating effect of inhibiting VE-PTP.[1,4]

In conditions such as diabetic retinopathy (DR) and AMD, where hypoxia plays a role, VE-PTP is upregulated, continually dephosphorylating Tie2; and if VE-PTP is active enough, it will be impossible to overcome it by extracellular agonists. That is, Ang-1 becomes ineffective with sufficient upregulation of VE-PTP.[1,4] So really, the only way to deal with this—Ang-1 resistance, if you will—is to suppress VE-PTP.

THE TIE2 PATHWAY IN OCULAR DISEASE

DUGEL Let me now ask about the clinical implications of what we have heard so far. Regardless of the particular strategy used, where might a Tie2 activator be most useful?

JEFFREY S. HEIER, MD The potential importance of manipulating the Tie2 pathway is significant. We have seen that blocking the VEGF pathway is effective in a majority of patients with diseases like AMD or retinal vein occlusion, though there remain patients with these diseases who do not respond well to anti-VEGF treatments.[9,10] But there is one disease state in which, while anti-VEGF therapy has been highly effective, it still leaves much to be desired: DR, and in particular DME.[1]

Vascular destabilization is paramount in DR and DME.[3] And we know that in addition to VEGF, many factors, cytokines, and chemokines are elevated in DR, including Ang-2.[3,11,12] Indeed, the Ang-2 / Ang-1 ratio is elevated in patients with DR in all forms, as shown in samples taken from vitrectomy.[3,8,13,14]

In diabetic patients, elevated Ang-2 appears to play a couple of different roles in vascular destabilization. In the absence of hypoxia and elevated VEGF, elevated Ang-2 leads to vasoregression, the process of progressive capillary destabilization which is thought to be the first stage of many microvascular diseases, including DR.[3] When both Ang-2 and VEGF are elevated, as is the case with hyperglycemia and hypoxia, the result is neovascularization (Figure 2).[3]

CAMPOCHIARO I think Dr. Heier summarized that very well. The Tie2 pathway seems to be involved at every stage in the pathophysiology of progressive DR. It has been demonstrated that

hyperglycemia causes upregulation of Ang-2, and Ang-2 has been implicated in pericyte apoptosis.[3] As the disease progresses, hypoxia also upregulates Ang-2, and VE-PTP further destabilizing the vasculature and increasing its responsiveness to VEGF—which, in turn, promotes leakage and the development of neovascularization. So, certainly, the ability to modulate this pathway pharmacologically has huge potential for DR and DME.

But we should not underestimate its potential for neovascular AMD. I agree that VEGF inhibition treats the majority of patients with neovascular AMD quite well, but problems remain. We know that long-term treatment with anti-VEGF agents can result in regression of neovascularization and even geographic atrophy in some patients.[9]

This suggests that some of the neovascularization, or some types of neovascularization, may be adaptive and are functioning to supply oxygen and nutrients to stressed tissue. By stabilizing the vasculature, through a combination of both suppressing VEGF and activating Tie2, it may be possible to have those vessels mature, be less likely to regress over time, and perhaps take advantage of whatever adaptive features they may have.

HEIER Those are good points in thinking about AMD. Some of the early, pre-clinical work on Tie2 pathway-modifying agents has shown a benefit of combination therapy in models that are representative of angiogenesis and neovascularization.[4,8] That is, the combination of an anti-VEGF agent and a Tie2 activator was superior, in terms of both prevention and regression, to either agent alone.[4,8] And when we look at early-phase studies in humans, there is at least a trend towards greater durability of effect.[15]

> Blocking VE-PTP leads to Tie2 phosphorylation irrespective of the level of Ang-1. This is a very important point, because in conditions like diabetes or AMD, where there are elevated levels of Ang-2, there tend to be low levels of Ang-1 in the extracellular space.

KAISER We could reasonably expect a benefit from activating Tie2 by itself. But when Tie2 is activated, there appears to be a process complementary to VEGF inhibition at work. Both monotherapy or combination therapy using this pathway have scientific rationale.

CAMPOCHIARO I certainly agree, and in the pre-clinical models that have been investigated, it is very clear that activating Tie2 by blocking VE-PTP was beneficial on its own.[4,5] The small-molecule VE-PTP inhibitor, AKB-9778, was effective in suppressing neovascularization and VEGF-induced vascular leakage in mouse models.[4] But humans are a bit more complex than mice.

The TIME 1 (Tie2 Activation for Treatment of Diabetic Macular Edema 1) study assessed the tolerability and pharmacokinetics of AKB-9778 in patients with DME, using four dose cohorts (5 mg, 15 mg, 22.5 mg, or 30 mg, administered subcutaneously BID) of six patients each.[16] In several patients, monotherapy with AKB-9778 suppressed vascular leakage quite well.

Among those receiving 15 mg BID, four out of six patients had a very substantial reduction in DME (reduction of 50 µm or more in central subfield thickness [CST], as measured by spectral-domain optical coherence tomography).[16] But at higher doses, fewer patients had this substantial reduction in DME (one patient in the 22.5-mg cohort, and two in the 30-mg cohort). And we really did not understand that heterogeneity. It was important going into the TIME 2 study to work out whether AKB-9778 could be used as a monotherapy for DME or would have to be combined with VEGF suppression.

The TIME 2 study randomized 144 subjects with DME to (1) monotherapy with AKB-9778 (15 mg subcutaneously BID) and monthly intraocular sham injection; (2) combination therapy with AKB-9778 and monthly ranibizumab 0.3 mg; or (3) monotherapy with monthly ranibizumab 0.3 mg and subcutaneous sham injection BID.[15] The primary outcome was mean change in CST from baseline at week 12; other measures included best corrected visual acuity (BCVA), safety, and Diabetic Retinopathy Severity Score (DRSS).

In TIME 2, the effect of monotherapy with AKB-9778 was less pronounced than in TIME 1, but the combination with ranibizumab was significantly better at reducing DME than either agent alone.[15] Interestingly, monotherapy with AKB-9778 did have a substantial effect on DRSS. In the ranibizumab-only group, 24 of 47 fellow eyes had background DR that was not treated

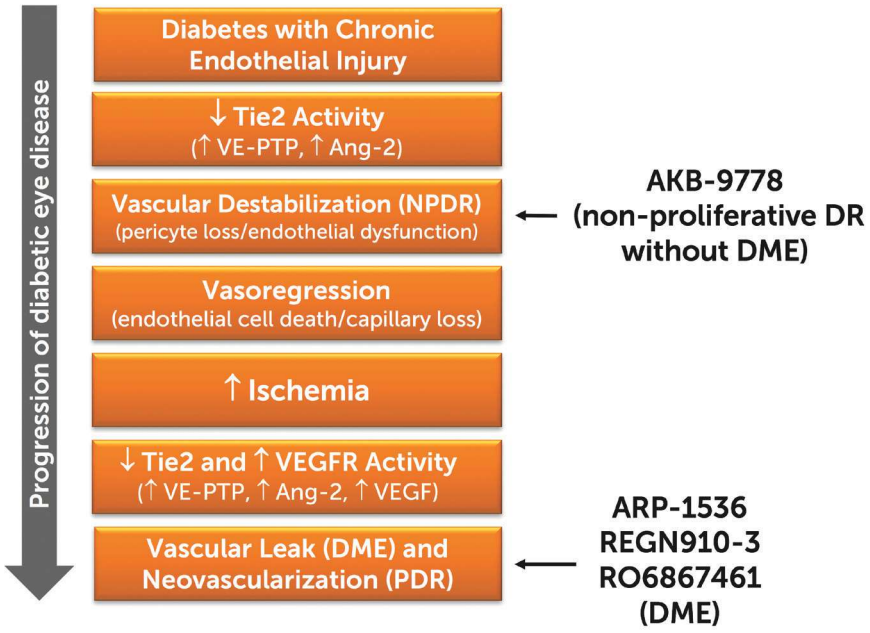

FIGURE 2 The role of the Tie2 pathway and investigational agents along the progressive course of diabetic eye disease.

locally with anti-VEGF or systemically with AKB-9778; of these, 4.2% showed ≥ 2-step improvement in DRSS. Among fellow-eyes in the AKB-9778 monotherapy and combination groups, 70 of 94 had background DR that was not locally treated, and 11.4% showed ≥ 2-step improvement in DRSS (Figure 3).[15]

This suggests that monotherapy with a Tie2 activator might have a role in treating non-proliferative DR.

PHARMACEUTICAL APPROACHES TO THE TIE2 PATHWAY

DUGEL Let's talk about the drugs that are in clinical trials right now (Table I). We described two approaches to activating Tie2. One is AKB-9778, which, as we have discussed, inhibits VE-PTP.

Two other products under investigation take the strategy of inhibiting Ang-2. One is REGN910-3 (Regeneron), a coformulation of the Ang-2 inhibitor nesvacumab and the VEGF inhibitor aflibercept. The other is RO6867461 (Roche – Genentech), a bi-specific antibody developed using CrossMAb ("crossed" monoclonal antibody) technology, with two fragment-antigen binding (Fab) arms, one inhibiting Ang-2, the other VEGF.[8]

Given that the trials are currently ongoing, let's begin by talking about the Regeneron coformulation. Dr. Heier, you have presented some positive results on this product. Could you discuss them?

HEIER We did an early-phase study looking at different doses of the anti–Ang-2 monoclonal antibody in combination with aflibercept (standard dose) in patients with AMD or DME.[17,18]

First, it is important to bear in mind that the subjects had been previously treated with anti-VEGF agents other than aflibercept. I tend to discount the visual acuity data in such studies, because the number of patients is small, they have been treated before, and aflibercept is itself an outstanding anti-VEGF agent.

We first wanted to gauge, is the formulation safe? And in that study, it appeared to be safe.[17] Secondly, are there any biologic signals to be gleaned from the study?

As I briefly mentioned before, at higher doses of the anti–Ang-2 agent, we started to see what appeared to be a trend towards greater durability of the treatment-free interval.[17]

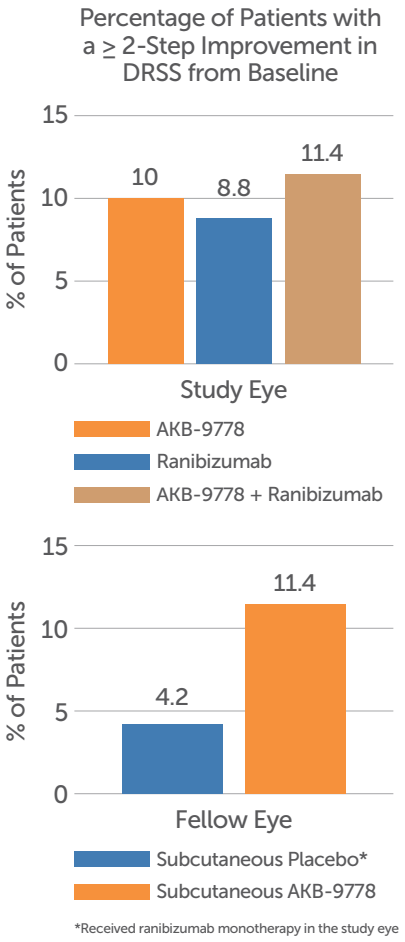

FIGURE 3 Improvement (≥2 steps) in DRSS after 3 months of treatment in study and fellow eyes with nonproliferative DR at baseline. Ranibizumab 0.3 mg was dosed monthly by intravitreal injection; AKB-9778 15 mg was dosed BID subcutaneously.[15]

Two large (over 300 patients), phase 2 studies of REGN910-3, ONYX and RUBY, are underway, comparing the efficacy of the anti–Ang-2 / aflibercept combination with aflibercept alone in neovascular AMD and DME, respectively.[19,20]

DUGEL The other agent, the bi-specific RO6867461, is a very elegantly-designed molecule with two Fab arms, inhibiting Ang-2 and VEGF. There is an Fc component, with two mutations in the FcRN and FcR-gamma foci that decrease the systemic circulation and eliminate the effector cell function.[8]

The ongoing phase 2 studies for this product are AVENUE for choroidal neovascularization in AMD, BOULEVARD for DME, and STAIRWAY to study the durability in neovascular AMD.[21-23]

Dr. Kaiser, what do you expect from these trials—increased efficacy, durability, or both? What do you think are the parameters for success?

KAISER Based on what we have talked about here, and on the science of both the Tie2 and VEGF pathways, we would hope that this agent will demonstrate improvements in visual acuity and/or OCT findings. Durability is a function of many things, including half-life, so whether either anti–Ang-2 / anti-VEGF agent will increase the durability of aflibercept or ranibizumab, is hard to say.

HEIER Yes, and not only are the ongoing phase 2 studies large enough to give us a signal, but in ONYX, the neovascular AMD trial of REGN910-3, the patients are treatment-naïve, which should also give us a good indication of how the combination works versus the VEGF inhibitor alone.[19]

As far as measures of success, there are three components we always look for: safety, efficacy, and durability. As a baseline for safety, anti-VEGF agents themselves are relatively safe: even understanding that the risk of endophthalmitis is real, the reported rates now are roughly 1 in 3000.[24] The systemic side effects, such as hypertension and thromboembolic events, are certainly something we consider, but we would need huge studies to show signals.[9]

In terms of durability, we have agents right now that allow for 4, 6, or 8 weeks between injections for most patients; some patients get 10 or 12 weeks. So I would look for the ability to extend at least quarterly—12 weeks or longer. And we may have an agent with that potential right now: top-line reports about Novartis's anti-VEGF agent brolucizumab show quarterly durability with matched efficacy.[25]

As for efficacy, to really have something meaningful, at a minimum I would look for three to four letters more improvement than with an anti-VEGF agent alone.

CAMPOCHIARO I would agree that increased durability would be desirable. In order for either combination to be approved, it really would have to show some superior efficacy over anti-VEGF alone. The FDA has not provided specific

TABLE I Investigational drugs targeting the Tie2 pathway

Drug	Details	Administration	Stage of Research
AKB-9778 (Aerpio)	Small-molecule VE-PTP inhibitor	Subcutaneous injection BID	Phase 2b studies in DR
ARP-1536 (Aerpio)	Anti–VE-PTP antibody in combination with anti-VEGF therapy	Intravitreal injection	Pre-clinical
REGN910-3 (Regeneron)	Anti–Ang-2 antibody in combination with aflibercept	Intravitreal injection	Phase 2 studies in neovascular AMD and DME
RO6867461 (Roche – Genentech)	Bi-specific anti–Ang-2 and anti-VEGF antibody, developed with CrossMAb technology	Intravitreal injection	Phase 2 studies in neovascular AMD and DME

guidelines, but rather will look at the totality of all outcomes. In terms of BCVA, an improvement of three to four letters over anti-VEGF would probably be a reasonable guide.

DUGEL It sounds like the bar is quite high for Ang-2 inhibition in neovascular AMD, because we have done so well with VEGF suppression alone.

Let's turn to the other Tie2 activation strategy, which is the inhibition of VE-PTP. That is the strategy Aerpio has taken with their agent, AKB-9778, with which subcutaneous administration also has the potential advantage of treating both eyes. The company has decided to pivot from targeting DME to targeting DR. It is one of the few companies I have known to change their strategy from one disease state—within the same overall disease, of course—to another. What are your thoughts about that?

HEIER I think it is an extremely wise approach, for two reasons. First is the question of FDA approval: as Dr. Campochiaro explained, TIME 2 showed us that to really see a benefit in DME, a combination agent is most likely needed. That means that to achieve an approvable drug, they need superiority—a much higher hurdle to clear.

But targeting DR, where monotherapy is likely to be a successful strategy, represents an easier path to approval. Moreover, the need and potential market for a DR treatment are huge.[26] DR is also typically more bilateral than DME, in which one eye is often more severely affected than the other. AKB-9778 represents an opportunity to treat both eyes at once, and there may be other systemic benefits of vascular stabilization. Finally, once approved, the drug could be combined with VEGF inhibition where appropriate, such as for the management of DME.

KAISER I agree that a treatment for DR may have a much greater impact than one for DME. It has been estimated that only about 7% to 10% of patients with diabetes have DME; but the majority, especially over time, will develop retinopathy.[26] One could even argue that improving DR should eventually reduce the incidence of DME and other long-term consequences of diabetic eye disease.

Further, systemic administration may have the opportunity to improve diabetic complications throughout the body—and in particular, the kidneys.[26-28]

Aerpio also has a humanized monoclonal antibody to VE-PTP in pre-clinical testing that offers intravitreal administration. If combination therapy is what is ultimately needed for DME and wet AMD, that agent makes sense for patients already committed to intravitreal injections of anti-VEGF agents.

Dr. Heier also mentioned the potential benefit to the kidney. So many of our patients with diabetes have proteinuria, which provides a fairly good biomarker for diabetic kidney disease, and in the ongoing trial for AKB-9778 in DR, the investigators will also be assessing proteinuria.

DUGEL Given all this—that VE-PTP is a relatively downstream target, independent of Ang-1 and Ang-2, and that a subcutaneous administration has a host of potential benefits—what are some parameters for success with AKB-9778 in DR?

CAMPOCHIARO I think the FDA will look for the percentage of patients who get a 2-step improvement in DRSS. Generally, there is a very small baseline rate (about 3% to 4%) of patients getting a 2-step improvement in DRSS, for miscellaneous reasons such as improved glycemic control.[15] In the TIME 2 trial, it was about 11.4% for patients receiving AKB-9778—approximately a three-fold improvement over the baseline rate.[15] A two-to-three-fold jump in the number of patients with a 2-step improvement in DRSS would likely be considered a clinically significant beneficial outcome.

> If VE-PTP is active enough, it will be impossible to overcome it by extracellular agonists. That is, Ang-1 becomes ineffective with sufficient upregulation of VE-PTP. So really, the only way to deal with this—Ang-1 resistance, if you will—is to suppress VE-PTP.

CAMPOCHIARO By activating endothelial nitric oxide synthase, AKB-9778 does cause some vascular dilation, and as a result, the TIME studies have shown a small but pretty reproducible reduction in blood pressure.[1,15,16] In general, any reduction in blood pressure, as long as it is not excessive enough to cause orthostatic hypotension, is a potential benefit.

Over time, decreasing blood pressure by even a few mm Hg can substantially reduce the risk of stroke.[29] And interestingly enough, that could perhaps provide some protection with VEGF co-administration, to counter the possible hypertensive effect of VEGF suppression.

HEIER The bar for success of a VE-PTP inhibitor in DR would only be lower than that for the Ang-2 approaches if the side-effect profile supported it. As we discussed, the side-effect profile of

intravitreal injections is really quite good now, but the very nature of intravitreal administration is somewhat invasive. If AKB-9778 is found to have a favorable side effect profile, in addition to the possible systemic benefits we discussed, that could be a very good reason to lower the threshold, so that maybe ophthalmologists would accept 50% of the benefit we would demand of an intravitreal injection.

KAISER But we also know that intravitreal injections are not sustainable, especially in certain patient populations. Even patients with exudative AMD—where missing a few injections risks permanent vision loss—have difficulty returning for monthly or bi-monthly injections.[30,31] The working-aged patient with DR but no DME and 20/20 vision is very unlikely to come in for anti-VEGF injections on a fixed repetitive schedule.

HEIER We know that there are many patients with diabetes who are at risk of vision loss due to varying levels of retinopathy, and obviously it is difficult to envision administering intravitreal injections for the years, if not decades, they might spend in this at-risk state. The potential ease of administration and systemic benefits of AKB-9778 could be extremely helpful, especially in those underdeveloped areas outside the US that already have access to insulin.

KAISER The chief complications of DR—DME and neovascularization—could potentially both be prevented by early intervention with subcutaneous administration of AKB-9778. We can even envision a time where, especially outside the US, the internal medicine doctor would prescribe this, not necessarily the retina specialist or even the general ophthalmologist. In doing so, we could reduce the incidence of DME, neovascularization, and thereby blindness—which would be a tremendous public health improvement.

And the combination VEGF and Ang-2 inhibitors we discussed earlier are exciting as well, because we are certainly still going to have patients with DME. If we have a better treatment than anti-VEGF monotherapy, which is, in my mind, the gold standard for DME, it is only good for all our patients, and for healthcare in general.

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