As a basic scientist it’s always exciting when we are able to team up with a clinician and really see if what we’re working on at the bench side is relevant in a clinical situation. So that’s something Ian and I have been partnering on for the last year I would say.

Just to give you an overview of the lab in our focus we are interested in fibrosis or the healer response basically when it goes wrong, so wound healing. And part of that is the angiogenic response, angiogenesis. Though it is systemically relevant when we think about wounds we think about the balance of angiogenesis. There is a up regulation early on in wound repair and then you want to have that inhibition to occur or a angiogenic pathological condition will result. And these are just to show you a few instances where physiological and pathological defects that occur because of the angiogenic state.

So just a brief overview of some of the inhibitors and regulators of angiogenesis. So there are angiogenesis inhibitors and promoters. Some of the promoters are VEGF or TGF alpha you may have heard of that. And when there’s dysregulation with these type of promoters with these type of growth factors you have excessive angiogenesis. To interest to us that excessive angiogenesis results in a fibrotic state which ultimately results in weakened skin and is also involved in other pathological situations such as tumor growth or diabetic neuropathy.

On the other hand you have inhibitors of angiogenesis and they can also cause a defect as well where there’s insufficient amounts of vascularity and we see this in heart disease and in a wound healing
case ulcers, whether they’re diabetic ulcers or venous ulcers. So these 2 type of inhibitors and promoters of angiogenesis have to work in a perfect balance, it’s a very delicate balance. Therapeutically there have been some headway that has actually in research that has actually made it into the clinical situation. One of the problems with that though they are targeted approaches, they are interested in interrupting the different types of cell signaling pathways, they use, we use inhibitors now to inhibit growth factors such as VEGF which I briefly mentioned, and then there’s indirect inhibition. Avastin is something that Ann has told me that you all use in the clinic, but some of the problems they’re either too targeted or they’re not targeted enough, or they only modulate one aspect that is contributing to the angiogenic state.

And our interest is in to Chemokines are these small cytokines and they’re shown to be more ubiquitous in nature in which they modulate angiogenesis throughout the entire healing process from the time where it needs to be promoted to repopulate the wound environment up until the point where there needs to be inhibition to prevent persistence. And of these chemokines we’re interested in the CXCR3 family and it’s just one of the chemokines that we’ve shown recently regulates the fibrotic state. And not only in the early stages of inflammation but also late stages where it inhibits the endothelial cell migration and fibroblasts into the wound to reduce the angiogenesis or the new development or the development of new neovascular vessels.

Just to give you a little bit of background on CXCR3, it’s a 7 transmembrane, chemo receptor, G protein receptor and depending on which group you’re talking to it has 4 lignins and the name of
those lignins differ so you may have heard of CXCL10, CXCL11, CXCL4 AND CXCL9. I will refer to them as IP10 and IP9, also there’s platelet factor 4 which is the CXCL4 and platelets sorry MIG which is CXCL9. But however, what’s interesting to us and we’ve mapped out the signaling pathway over the last 10 years but what is most interesting about this receptor is its ubiquitous nature. In a wound healing state it promotes the migration of keratinocytes, leukocytes to infiltrate the wound. At the same time it has the inhibition or this inhibitory nature in which it can block growth factor induced endothelial cells and fibroblast migration into the wound via ____ and that’s huge. In growth factors I mean VEGF, EGF, PDGF alpha, and others.

So because IP10 of all of these which is also referred to as CXCL10 has been shown to be anti-angiogenic we’ve looked further at it’s role within the ciliary system in a wound healing context. It is found to bind only to the CXCR3 receptor speaking to its specificity, it’s secreted by a host of cells, keratinocytes, leukocytes but most importantly to us is the fibroblast into endothelial cells and it elicits the migration as I mentioned of keratinocytes yet inhibits that of fibroblast and endothelial cells into the wound.

So some of our preliminary studies or earlier studies showed that IP10 inhibits the formation of vessels. In vitro we were able to show using human microvascular endothelial cells that after inducing vessels or stimulating these tubes which we call in vitro with VEGF, IP10 is able to block that formation of those tubes. And in the in vivo state where we use matrigel plugs and we inject them into the mice that are supplemented with VEGF to form these vessels in the plug, IP10 was
able to block that vessel formation. But more importantly as we think therapeutically IP10 was also able to cause a regression of these newly formed vessels. So this experiment was done slightly different in which we allowed the vessels to form first after 10 days we injected the matrigel plug supplemented with VEGF, vessels were able to form and then we administered a dose of IP10 or saline and we allowed that to go out for 7 additional days. As you can see the saline or the untreated situation there still remains a vascular network within the plug, however, with IP10 you can see that these newly formed vessels begin to disassociate. So this type of association or aggression is important when we’re thinking of this neovascular state.

So as I mentioned earlier there is a ubiquitous nature in regard to the CXCR3 signaling system and the chemokines that are regulated that it regulates. So we decided to look at the mode in which CXCR3, I mean IP10 binds the CXCR3 and caused this inhibition and we were able to identify a particular region within the full length of IP10 an developed an IP10 peptide. It’s a smaller molecule and we believe that we thought that by doing this we could have a more targeted approach and also induce a greater level of specificity.

So we did the typical kinetics and binding studies on the IP10 peptides to see if it binds to endothelial cells in a similar fashion that the full length did, and it did. It also blocked the migration of VEGF induced endothelial cell migration after in a similar fashion in which IP10 blocked the migration. And so we performed similar in vivo studies to look at not only the inhibition of IP10 peptide to be able to block VEGF induced vessels but also cause that regression. So in a very similar
plug assay within the ____ we induced these plugs to form vessels with VEGF and IP10 was able to inhibit that formation. On the other hand well in addition to that we induced these plugs to form these vascular networks after 10 days the networks were formed, then we added IP10. Seven days later or day 17 you can see here that IP10 was able to cause the regression of these newly formed vessels.

And just as a confirmation study we wanted to, we used CD31 to identify that these are actually vessels within the plug and that they’re immature vessels we used a desmin stain and we were able to show that. So there’s a host of therapeutic opportunities where we’re looking at inhibiting angiogenesis and to date there has been a strategy. Some of the identifiers in this strategy is the inhibition of endogenous proangiogenic factors such as the VEGF that I’ve mentioned or the degradation of or inhibiting the degradation of enzymes. It should inhibit the endothelial cell proliferation and migration and most importantly cause the or inhibit the activation or differentiation of endothelial cells. So when we look at that strategy and compare it to some of the properties of the IP10 peptide we are shown that IP10 peptide is able to block the endogenous proangiogenic factor such as VEGF. It also inhibits endothelial cell migration and vessel formation and most importantly to us we think that it has the ability to directly cause the disassociation and regression of these newly formed vessels.

So that leaves us with the question, is this clinically relevant? And this is when I will turn it over to Ian.
So to summarize then up to this point we have I think a very interesting and novel peptide agent that works at various stages within the wound healing response, has been demonstrated in smaller species and in ex vivo studies to have these properties. And as an investigator as a young faculty member in a place like this, it’s very exciting because I get the opportunity to interact with folks who are really devoting all their time and their passion into developing strategies like this but then come to me or come to investigators like us, surgeons, clinicians with the question you know what kind of model could we have or could we think of or exists already that we could use to investigate these properties further in a way that is more directly clinically relevant and lends itself to the idea of direct translation from the bench to the bedside and that’s really the point of this session as well is to think about bench to bedside science.

So obviously I’m a glaucoma specialist and I perform filtration surgery or fistulization surgery all the time, I don’t have to explain how aqueous flows within the eye to the folks in this room so we’ll move ahead but we’ll come back to that in just a moment. So wound healing in all surgery, not just glaucoma surgery, is discussed to have essentially 3 phases in normal wound healing. There’s the inflammatory phase which is dominated by white blood cells, cytokines, blood clot that’s sort of this portion of the figure here in the sequence of events in wound healing chart. Then there’s the proliferative phase which is dominated by fibroblast activity extracellular matrix formation in the angiogenesis. And then a remodelling phase which is dominated by scar formation. So obviously these are all issues that we should all understand and that are very important in the kind of surgery
that I do. To think about this clinically I make blebs, none of us I think like blebs but they’re a necessary evil in glaucoma surgery, so I make blebs and we see good blebs and we see bad blebs and sometimes they’re secondary to the things that we do at the time of surgery in order to try to encourage bleb formation and bleb retention over time. So there’s successful blebs, good blebs could be you know shallow and diffuse, they could be somewhat focal and cystic but still functioning, they can be completely scarred down that’s a bad bleb, or they can become encapsulated, that ring of steel that we all think about and fear in which case they’re just an extension of the anterior chamber and not particularly effective. So these are, these are guided wound healing that went well and guided wound healing that did not go so well. Because fundamentally our job as glaucoma surgeons or anybody who does glaucoma surgery is to create a situation of incomplete wound healing. So we do that by modulating the wound healing response with various agents both administered at the time of surgery and afterwards. So we modulate the inflammatory phase of wound healing with corticosteroids, we modulate the proliferative phase of wound healing with mitomycin C and 5 FU the anti fibrotics that arrest the cell cycle, and we modulate the proliferative phase especially the angiogenic phase with anti VEGF agents. This has been looked at in glaucoma surgery using anti VEGF agents like Bevacizumab, Ranibizumab both in the clinic as well as in animals with some success, some moderate success certainly seems to have a profound affect on angiogenesis. I think the jury is still out on these agents as far as the long term utility toward bleb modeling I guess I would say and function.
But much more commonly we use Mitomycin C, so again we’re all familiar with the toxic effects of Mitomycin C. Before Mitomycin C blebs all failed, after Mitomycin C many of them do not fail over a long period of time. However the gift of Mitomycin C is one that keeps giving over a long enough period of time sometimes we see these blebs that become really thin, avascular, cystic, and can even then develop infection, blebitis and ophthalmitis. You can have over filtration leading to hypotony and hypotony maculopathy and loss of vision, bleb leaks, ciliary body toxicity has been reported, scleritis, scleromalacia and endothelial cell loss have all been reported as well. And this figure just shows fibroblast proliferation in eyes that received, this is, well not in eyes in ex vivo, in slides so controlled fibroblasts on a plate. Mitomycin arrests the cell cycle and really just wipes them all out forever and ever, 5FU has that effect but much less significantly. So again we’re interested in ways that as clinicians and surgeons we can help in these endeavors to develop new agents that modify wound healing and angiogenesis and the model that I was able to propose with Cecelia is a rabbit model of trabeculectomy, actually it’s sort of a hybrid between a rabbit model of trabeculectomy and a tube shunt because I don’t actually perform any ectomy I don’t remove any trabecular tissue but I do use a 22 gauge Angiocath as a tube shunt to hold open a patent fistula between the anterior chamber and the subconjunctival space, so this is a New Zealand white rabbit and essentially in the supratemporal quadrant I form a peritomy, I use a 23 gauge needle just like I would use in humans when I implant tube shunts or setons to tunnel into the anterior chamber through the sclera. I insert a beveled 22 gauge Angiocath into that incision and then cut the Angiocath flush with the sclera after I’ve sutured it down to the sclera using 10.0 nylon and then I close the peritomy with 10.0 nylon ensure that I have a watertight incision in the bleb form and that’s
that. The entire thing usually takes 20-30 minutes per eye. So what you are left with then, this is a rabbit on it’s in the first week I forget exactly what day but this is a nice superior bleb in this animal. Now none of these animals of course have glaucoma they’re just New Zealand white rabbits you now straight from the vendor.

So the experiment that we devised was based on prior literature as far as surgical technique goes and power but we chose to study or use 10 rabbits, treat 10 with the peptide so Cecilia spent some time talking about the peptide versus the full length protein. The peptide appears to have all of the functional properties as the full length protein and perhaps even be a little more effective because it’s a better fit with the receptor. We completed 10 surgeries using the peptide 10 using the full length, I did perform bilateral surgery and there’s a very good reason for this, so the right eye was treated with the agent and the left eye was treated with BSS injections instead of the agent, and the reason is that this provides an internal negative control for the surgery. So I performed exactly the same surgery on both eyes, but one eye is treated with the test agent and the other is not. In addition we can look at conjunctiva within the eye away from the area of surgery at the time of sacrifice so we can look at the conjunctiva inferior leak for example, we did do that as the second negative control. I do have plans as well to do a positive control with Mitomycin but we’ve not gotten to that yet. And I included here because it’s always a question about this with bilateral surgery a lot of folks have a conception that the ARVO guidelines disallow bilateral surgery because it is considered I forget exactly the term but it’s a potentially visually disabling condition, so the ARVO guidelines actually say that they recommend that animals are not subjected to multiple major survival
procedures so this is one surgery not multiple surgeries but it is bilateral major survival surgical procedure, unless they are related components of a particular research project so I felt like the fact that we could have a negative control that is perfectly controlled compared with the other eye because we don’t know what the systemic effects of the IP-10 peptide could be, was justification enough and the IACUC, the IACUC here agreed with me that that was a reasonable thing to do because they are related components of the same specific goal. So you are allowed to do bilateral surgery if you have a good justification for doing it. So anyway, so the bilateral surgery was performed. At the time of surgery the subconjunctival injection of the test agent was given as well as the subconjunctival injection of BSS to the alternate eye and then on days 2, 4, and 7 so four injections total they received the test agent as well into the bleb. A weekly exam was performed including an IOP check using a rebound tonometer and photographs and then sacrifice in histopathology were performed at post op week 6. So the results the first thing you notice is that there’s no difference in IOP over the course of the 6 weeks or 5 weeks here noted and that’s not surprising these are animals that didn’t have glaucoma to begin with, all of their blebs did fail by the end of the test period subjectively there was a better bleb survival in the animals that received the peptide, but they all failed within 2-3 weeks. And a problem in our study is that we didn’t have an independent observation so it was just me and you know I could be biased. I suspect I am but I also can tell you subjectively that there was a difference, that’s something we’ll remedy in the future. But there were significant differences noted in inflammation, angiogenesis, collagen deposition and conjunctival goblet cells which is interesting. So I’ll show that data in just a moment. So again we have a non-injured site so let’s see inferior conjunctival of each eye, the control sites so this is the eye
that was operated on but didn’t receive the test agent and then full length and peptide agent so we had increased signs of inflammation in the control agent or in the control condition compared with the full length of the peptide we had increase in thickness of fibro layers in the control and increase in fibrosis and these were modulated by the full length and peptide IP-10, so this is where it really gets interesting. So we expected a decrease in the angiogenesis in the treated animals and indeed we saw that so in the control animals that did not receive a peptide there was a significant increase in angiogenesis into the area of the bleb. In the animals that were treated especially the peptide animal, essentially angiogenesis was arrested through administration of these agents. There’s decreased fibrosis within the treated blebs so again we have the control untreated with the peptide agent had significant fibrosis which was blunted markedly in the treated animals. And then cellularity within the bleb area was also decreased in the treated animals so we have overall we’re seeing a decrease in healing, aggressive healing response.

And then finally this was an interesting finding that we didn’t really expect but we assessed nonetheless. There was actually an increase in goblet cell counts within the conjunctiva epithelium overlying the bleb in treated eyes compared with untreated eyes. So it’s been previously published in the literature in rabbits the average goblet cell count is around 7 or so and that’s what we saw in the uninjured site. And our control site we saw a significant decrease in the number of goblet cells, but then with treatment we actually saw an increase in the number of goblet cells. As you all know who treat glaucoma or take care of patients who’ve had glaucoma surgery previously or patients who have not had surgery but are on topical agents, dry eyes is a significant issue and a lot of the reason
we believe in this has been demonstrated in the literature, has to do with toxicity to the conjunctival epithelium and loss of goblet cells, loss of mucin production. So this is a potentially, it’s a surprising but potentially very interesting and useful finding as we go forward and think about potential applications of this agent. So you’ve seen this slide before with Cecelia a few minutes ago and I just threw it up again to emphasize that we are treating this CXCR3 receptor system with a selective binding agent IP-10 and that we would expect it to have effects both in the early inflammation phase, in the later fibrosis and angiogenesis phases and potentially even in the wound remodeling phase because we know that CXCR3 receptors are involved in all 3, all of these phases of wound healing.

So the next steps in our project because I think we have some really exciting pilot data here, is first to establish and active control so I need to perform the surgery on a number of rabbits using Mitomycin C and then compare them because I would expect to see you know a decrease in a lot of these measures and fibrosis and in the cellularity and everything as well as in the goblet cell count. It’s been published that the goblet cell counts in animals that have received Mitomycin C blebs is around 1 per high powered field. So a really marked decrease but it’s important for the purpose of moving forward and considering publication. As we move into the next phase and consider further experiments involving this surgery I plan to utilize a mass or blinded grader of bleb survival, so somebody other than me maybe I have a fellow who could help me with that. And then potentially utilize the surgical model with hypertensive animals. So these models do exist in multiple species including rabbits, and it would be interesting to utilize a model and look at bleb survival in those
animals who actually have ocular hypertension because the stress in on the bleb or very different than in animals that do not. We could consider alternative methods of administration in the future. Again these were subconjunctival injections which is possible but not entirely practical if you’re thinking about translating this type of finding into the clinic. So we could consider dropper administration or some sort of implant administration perhaps bound into a hydrogel or something like that. So there’s lots of exciting avenues for further experimentation.

And then finally I put here consider the serendipitous result, we didn’t expect this goblet cell finding but I think in the world of dry eye that is potentially a really big and important finding and one can imagine this type of agent being utilized to treat ocular surface disease or at least play a role in managing ocular surface disease in the future.

So I just have a few acknowledgements then, looks like we’re right on time despite the delay which is excellent. So Alan Wells is in the department of pathology, he’s I believe the assistant chair or the vice chair and Joel Schuman so you know who these people are. They’ve been incredibly supportive, they also have some intellectual property interest I think in this particular project, but have been you know very gracious and very helpful in funding and making this go forward. Cathy Yates is sitting here to my left, Eric Romanowski and Katie O’Connor, Cathy really especially was really amazing in helping get this project done both in managing the paperwork involved with the animals and helping me get the surgeries done. So she’s done a lot of this kind of work before as part of the Campbell Lab and I wouldn’t have known where to start without her. So she’s really
been amazing and I couldn’t have done the project without her. Mike Nakon is a surgical assistant or surgical technician in the DLAR, the Division of Laboratory Animal Research I think is what it stands for but over in the other tower or essentially in this tower upstairs, and really helped me to get acclimated to operating on animals so he was incredibly useful and knowledgeable. Megan Link is sitting behind me here, she’s an undergraduate student who is now our local expert in ocular histopathology through this project. Then Rachel Davis has left us for her first faculty job but she was our fellow last year and helped me with several of the surgeries when I was just unavailable because I was taking care of patients and that sort of thing. So that said I’m always interested in other volunteers if we have residents or fellows in the room who are interested in finding a project, this is but one of my several projects, I’d be happy to talk to you about ways that we could collaborate. That’s it.