I’m the New Division Chief in Pediatric Hem/Onc and I haven’t met a lot of you and I’m looking forward to getting to know you as I work more in the hospital and more with the residents and medical students. Today I’m going to be talking about B-cell lymphoma and how B-cell lymphoma can arise as a complex interplay of infection and inflammation and chromosomal translocation. And I hope that at the end of the talk I can convince you that understanding these mechanisms can lead to profound changes in the way that we talk to patients and treat patients.

Okay, so it’s long been known that infection can play a causative role in cancer. And there is many examples and I think these are some of the more famous examples, hepatitis and its association with hepatocellular carcinoma, h-pylori associated with gastro carcinoma, schistosomiasis with bladder cancer, etc. The way that infection causes cancer of course varies depending on the context and it’s extremely complicated. And one of the parts of this process is the fact that infection can induce a state of chronic inflammation and inflammation can then promote the process of tumor genesis. And on top of that there is a contribution of genetic abnormalities either just within the cancer cell or within the patient as a genetic predisposition that leads to this process.

In pediatric lymphomas the process of how infection and inflammation and genetic abnormalities interact is very complicated and very fascinating and has led to lots of exciting new discoveries. So in pediatrics lymphomas represent about 11% of childhood tumors making them the third most common type of pediatric cancer following leukemias and brain tumors. And lymphomas in kids can be divided generally speaking in about 50% Hodgkin’s disease and 50% non-Hodgkin’s disease.
We are lucky in pediatrics compared to in adult oncology because in adult oncology the categorization of non-Hodgkin’s lymphoma is extraordinarily complicated and there is at least 40 different types of cancers. In kids it’s much simpler and we can divide the types of lymphomas, non-Hodgkin’s lymphomas that happen based on the cell of origin. So you can have precursor cells either a precursor T or a precursor B-cell that can give rise to lymphoblastic lymphomas, and these type of lymphomas the cell of origin is very similar to if not identical to the blasts that are found in acute lymphoblastic leukemia, and in fact we treat these patients remarkably similarly. You can also develop non-Hodgkin’s lymphomas from mature cells either a T-cell or a B-cell. And in the case of T-cell one of the more common diagnosis in kids is anaplastic large cell lymphoma. The mature B-cell non-Hodgkin’s lymphomas are a fairly common group of diseases and they are composed of Burkitt’s lymphoma and diffuse large B-cell lymphoma, and then an entity called post-transplant lymphoproliferative disease and then there is a category of other more rare tumors which includes things like primary mediastinal lymphoma, follicular lymphoma and a disease that we study in our laboratory called MALT lymphoma.

So there are many, many infectious agents that have been associated with various types of non-Hodgkin’s lymphomas and this is just a partial list here including Epstein Barr Virus, Human Herpesvirus 8, HIV, etc. And in general when we talk about how infectious agents promote the process of lymphoma genesis we think about it in terms of 3 types of mechanisms. The first mechanism is the infection actually induces the transformation of the lymphocyte and causes it to
become a malignant cell. And the infectious agents that promote lymphoma in this mechanism include EBV, HHV, HTLV1. There is also a process by which infections such as HIV can induce cell-mediated immunodeficiency and being immunodeficient can put you at risk for a non-Hodgkin’s lymphoma and then finally there is a variety of infections that can induce a state of chronic stimulation and chronic inflammation and just that process can lead to the development of lymphoma.

So let’s first talk about the first mechanism, how lymphotrophic viruses can induce lymphoma. So the virus infects the lymphocyte, the virus then expresses certain viral oncogenes, particular proteins are expressed that have oncogenic properties, the cell undergoes a transformation and you develop a lymphoma. Of course this is a very simplified view.

And as I was contemplating whether I would come and take this job I did a lot of research in terms of what goes on at University of Pittsburgh and since this is a topic that’s near and dear to me I was really happy to see that the role of infection in inducing lymphoma is something that has a pretty high profile here at University of Pittsburgh and in fact this virus – well the KSHV virus was actually discovered by people here at UPCI, Moore and Chang, and a bunch of people here now study the role of this virus in the process of lymphoma genesis and there is many other examples including someone here at Children’s Hospital, Adam Rosendorf in the Pathology Dept. who studies EBV.
So Epstein Barr Virus is one of the most important causative infections in terms of pediatric non-Hodgkin’s lymphoma. It was discovered in 1964 by Anthony Epstein and it was actually isolated from cultured Burkitt lymphoma cells, it’s a member of the gamma subfamily of herpesviruses and it establishes a seemingly harmless latent infection in B-cells in over 95% of our population. And the question of why certain people develop an EBV driven lymphoma and others do not is an important and not completely understood question. So Epstein Barr Virus has been implicated in lots of lymphomas and I’m going to try to use this as a pointer since this isn’t working, oh now it’s working. Okay. So Epstein Barr Virus has been implicated in Burkitt’s lymphoma, Hodgkin’s lymphoma, post-transplant lymphoproliferative disease as well as some T and NK-cell lymphomas but in addition Epstein Barr Virus is implicated in other non-lymphoma cancers.

This is just a diagram of the EBV genome and when EBV infects a cell it can express various proteins that are encoded by the genes in this genome. And the genome includes what’s called the EBNA, the Epstein Barr Nuclear Antigens as well as the LMP genes which encode latent membrane protein, so these are proteins that then get embedded in the membrane of the lymphocyte that’s infected. So this is a diagram that sort of simplifies how Epstein Barr Virus ends up infecting our B-cells. So we can be exposed to the Epstein Barr Virus through the saliva. Epstein Barr Virus can then infect the oral mucosal epithelium and undergo lytic replication, it can make its way through the oral submucosa and come in contact with a B-cell. And it particularly has – it’s trophic for B-cells because it has a glycoprotein on its surface that recognizes a particular receptor protein called the CD21 protein, so it has a predilection for infecting B-cells specifically. And when it infects the B-
cell it then expresses certain proteins that are encoded on its genome and many of these proteins have oncogenic properties.

So the process by which EBV transforms a B-cell and contributes to lymphoma is very complicated, it depends on the circumstance and the picture that I’ve shown here is really somewhat of an oversimplification but I think it illustrates a couple of important points. So the EBV virus can recognize a specific protein on the B-cell surface, it then infects the B-cell and it expresses certain oncogenic proteins. Some of these proteins are LMP1, stands for latent membrane protein 1, and LMP2, latent membrane protein 2. And these cells are particularly interesting when it comes to the process of B-cell lymphoma. LMP1 is actually a mimic of a human protein called CD40 and CD40 is an important receptor protein on B-cells and when it is stimulated it promotes the proliferation of B-cells. When Epstein Barr Virus causes the expression of LMP1 in B-cells it’s as if the CD40 receptor protein is just constantly turned on so the B-cell is constantly stimulated and you end up with proliferation of the B-cell, and that is one of the ways it promotes oncogenesis. LMP2 shares some properties with the B-cell receptor itself and it kind of messes up the normal B-cell receptor signaling and promotes proliferation of the B-cell as well. And both of these can work by constantly activating an important transcription factor called NF kappa B. And NF kappa B is a transcription factor that normally resides in the cytoplasm but when it’s activated it can go to the nucleus and when it goes to the nucleus it activates a whole program of different target genes and drives the proliferation of the lymphocyte. And this is a normal and important response but when LMP1 and LMP2 are present because of an EBV infection it becomes out of control and too much NF kappa B
B-CELL LYMPHOGENESIS: A COMPLEX INTERPLAY OF INFECTION, INFLAMMATION AND CHROMOSOMAL TRANSLOCATION, LINDA M. McALLISTER-LUCAS, MD

activation leads to too much proliferation of the cell and contributes to the development of lymphoma. Now under normal circumstances this activated B-cell then proliferates but our own immune system with T-cells can keep this in check.

So Epstein Barr Virus has been linked to several lymphomas and which proteins are expressed in which lymphoma varies. So for example in Burkitt’s lymphoma most of the pathogenesis is driven by the nuclear antigen EBNA1 whereas in PTLD, post-transplant lymphoproliferative disease, the EBV expresses all of those proteins including those LMP proteins that I mentioned. So the mechanism by which EBV causes lymphoma varies depending on the disease.

So this is a picture of Burkitt’s lymphoma. This is one of pediatric hem/onc’s favorite diseases because it’s easy for us as not pathologists to recognize under the microscope, so we think we are super smart when we make the diagnosis before the pathologists do. And we see that there are these vacuoles in the malignant cells and then when you look at lower power it has this what’s called starry sky appearance, and I bring that up because this always appears on pediatric board exams. So Burkitt’s lymphoma in kids comes in two flavors, it comes as the sporadic North American form and the African endemic form which represents 50% of all childhood tumors in Africa, so it’s very, very common. In North America the most common presentation is an abdominal tumor whereas in Africa more commonly or almost exclusively it presents as jaw involvement. And the interesting thing here is that in the sporadic form EBV is present only in 15 to 30% of cases whereas in the African form EBV is present in 100% of cases. So this tells you that the relationship of EBV to the process of
lymphomagenesis is different depending on the context and that EBV is not absolutely required for the development of Burkitt’s lymphoma. So the question is how does it contribute to Burkitt lymphoma but not be required for Burkitt lymphoma? And I would say we don’t totally understand that yet.

But one thing that is required in Burkitt lymphoma is the presence of a chromosome translocation involving the protein myc, and many of you have heard about myc because we have big experts in our pediatric hem/onc faculty, Dr. Ed Prochownik and his trainee Tony Gregg study myc. So I hope I don’t say anything wrong about myc, and please forgive me if I do, Ed. So in Burkitt’s lymphoma you must have a translocation of the myc protein along with either the immunoglobulin heavy chain which is located on chromosome 14 or one of the immunoglobulin-like chains located on other chromosomes. When this chromosome translocation happens the gene encoding myc ends up adjacent to the immunoglobulin heavy chain gene, and the promoter of the immunoglobulin heavy chain gene drives overexpression of myc so you get too much myc and that messes with things.

So myc is a transcription factor that exists in complex with other proteins, it binds to the promoter of specific genes and it activates or I should say regulates the transcription. And there are really tons and tons of genes that are regulated by myc, it’s an incredibly important transcription factor. Many of the target genes regulate processings that are very essential to the normal growth of cells and if you have inappropriate levels of myc you can mess with this gene regulation and cause cancer,
specifically cause B-cell lymphoma. But in fact myc overexpression is implicated in many, many other cancers outside of B-cell non-Hodgkin’s lymphoma.

So c-myc is overexpressed because it’s gene ends up translocated adjacent to the immunoglobulin either heavy chain or the light chains leading to its deregulation of gene expression and too much myc. And it’s sort of the poster child for a process that commonly recurs in non-Hodgkin’s lymphoma, so there are many other examples of genes that end up translocated adjacent to this immunoglobulin heavy chain that’s found on chromosome 14, so you can see the recurring theme, this chromosome 14 or sometimes chromosome 2 or 22, those are the light chain genes. And the discovery of this as a recurring theme in B-cell non-Hodgkin’s lymphoma was really important because it helped us realize that these particular genes that end up translocated in these chromosome translocations have really important roles in regulating the growth of B-cells, and if they are involved in translocation things get screwed up and you get cancer. So surely they are very important. So c-myc is the most famous example.

Another famous example is BCL2, BCL2 is an antiapoptotic protein and if you make too much BCL2 because the BCL2 gene ends up translocated adjacent to the immunoglobulin heavy chain you essentially prevent apoptosis from happening, so you prevent cell death. That sounds like a bad thing to an oncologist and this is causative for the development of follicular lymphoma. And there is many other examples. Another interesting example is PAX5, a transcription factor that has a very important role in regulating the process of B-cell differentiation. If that gene is translocated
adjacent to the immunoglobulin heavy chain you get too much PAX5 and the whole program of B-cell differentiation is messed up and you end up with lymphoma.

So why is it that these translocations involving the immunoglobulin heavy chain are so common? It has to do with the fact of immunoglobulin processing, genetic processing in the – during the process of B-cell differentiation. So the B-cell precursors start their process of growing up in the bone marrow and they undergo a series of step from a progenitor B-cell to a mature B-cell. They are then released into the periphery and they undergo further differentiation in the lymph nodes and there are two processes that undergo, that happen called somatic hypermutation and class switch recombination, these are complex genetic events that allow us to make high affinity antibodies of different classes and when these very complicated processes go wrong you can get chromosome translocation.

So the myc IGH translocation or light chain translocation has to be present in order for Burkitt’s lymphoma to be diagnosed. So we have a situation in which c-myc translocation is required for the development of Burkitt’s lymphoma but EBV infection is only associated, highly associated with Burkitt’s lymphoma but is not absolutely required. So the question is what is the relationship here? And we don’t really understand fully but it seems that the EBV infection somehow promotes a permissive environment that allows myc driven tumors to form. And there is really two current theories. One is that EBV infection somehow increases the overall genetic instability allowing translocations to happen more frequently, or second that EBV infection can somehow potentiate the
effects of c-myc and allow lymphoma to proceed. And this is a drawing that illustrates those two theories. So without EBV we have the inherent genetic instability of the development B-cell, a chromosome translocation happens, c-myc is overexpressed and you develop a lymphoma. We know that has to happen in order for Burkitt’s lymphoma to occur. But if EBV is present how does that change things? Perhaps it results in more frequent events resulting in chromosome translocation occurring more frequently or earlier in the process, you end up with c-myc translocation and a B-cell lymphoma. Or perhaps the story is that an EBV infection actually accentuates the oncogenic effects of myc making it a more powerful oncoprotein.

One of the ways that EBV has been proposed to accentuate the effect of myc is illustrated in various or oversimplified terms here. So if myc is overexpressed myc happens to be very complicated and it can actually cause the proliferation of B-cells and that makes sense in terms of its oncogenic role, but also myc has the potential to drive apoptosis, and this is a very defined balance and it’s important to have proper regulation in order to balance these two processes. When EBV infects the cells some of its oncoproteins will be expressed and that can mess with the regulation of gene transcription and one of the genes that are targeted by EBV is BIM which is a proapoptotic protein. And in fact if BIM is not made enough because of EBV infection you end up with less apoptosis and then more proliferation. So there are some suggestions that this is just one of the mechanisms by which EBV and myc interact in the pathogenesis of myc driven lymphomas.
So the take home points on Burkitt lymphoma are that Burkitt’s lymphoma is associated with EBV infection, but EBV is not absolutely necessary nor is it sufficient to cause Burkitt’s lymphoma. C-myc translocation is necessary and the way these two events are related is not entirely understood but it’s believed that EBV infection somehow creates a permissive environment that allows myc to drive lymphomagenesis. So this is a great example of the complex interplay between an infection, EBV, a genetic abnormality, a translocation involving myc somehow contributing to the process of developing a cancer.

So now let’s move to the next category of non-Hodgkin’s lymphomas being caused by an infection and that is in the situation of HIV causing immunodeficiency. So HIV is associated with a large variety of non-Hodgkin’s – B-cell non-Hodgkin’s lymphomas and in general they are divided into three categories, the type of non-Hodgkin’s lymphomas that occur in other immunocompetent patients, excuse me that occur in immunocompetent patients as well as immunodeficient patients and that includes Burkitt’s lymphoma, diffuse large B-cell lymphoma and the disease we study in our lab which I’ll talk about later, MALT lymphoma. The second category is disease that occurs specifically in only the setting of an HIV infection and those include some rare cases of lymphoma that are driven by HHV8. And then the third category is lymphomas that occur in other states of immunodeficiency including post-transplant lymphoproliferative disease.

So how does this happen? Well AIDS is associated with a 60-fold increased risk of non-Hodgkin’s lymphoma, it often occurs in very unusual sites like outside of the lymph nodes and in the CNS.
of the cases of HIV associated lymphomas are also associated with EBV infection. So we are back at looking at this same picture I showed earlier of how EBV promotes B-cell transformation and we have – sorry, we have the EBV infecting the B-cell, the EBV then expresses its oncogenic proteins and causes proliferation of the infected cell. Along this process you can have the contribution of additional genetic changes that lead to frank transformation into a lymphoma and in this example they list diffuse large B-cell lymphoma. When our immune system is competent or normal we can in general keep this process in control because of EBV directed T-cells that stop the growth of these B-cells. But when a patient is HIV infected and is suffering from AIDS their T-cells don’t function properly and so they are at a much higher risk to go on to develop EBV driven lymphomas.

So this is an example of an even more complex interplay where we have an infection with HIV causing immunodeficiency which then allows EBV infections to take root and in addition the infectious processes can induce a state of chronic inflammation which we know we see with patients with chronic HIV, and what is the contribution of genetic abnormalities? Well for Burkitt’s we still need the c-myc translocation to occur in order for the lymphoma to occur. So this is a complex interplay of multiple infectious agents, immunodeficiency, a state of inflammation and chromosome translocation all contributing to the process of lymphoma.

So now we’ll talk about the final category here of mechanisms by which infectious agents can promote B-cell non-Hodgkin’s lymphoma and that’s by inducing a state of chronic inflammation. And the quintessential example of this is a disease called MALT lymphoma which is of course near
to dear in my heart because this is what we work on in part in our laboratory. So MALT lymphoma, MALT is an acronym, it stands for mucosa associated lymphoid tissue. MALT lymphoma is the most common form of extranodal lymphoma, that is it does not arise in the lymph nodes rather it arises in mucosal surfaces.

MALT lymphoma is associated with numerous infections. H-pylori has a strong association with gastric MALT lymphoma; Campylobacter with small intestinal MALT lymphoma; Chlamydia with ocular MALT lymphoma and Borrelia with cutaneous MALT lymphoma. In addition to infectious agents there is a number of chronic inflammatory diseases that are associated with MALT lymphoma. Hashimoto’s thyroiditis is associated with an increased risk of MALT lymphoma of the thyroid; Salivary gland inflammation is associated with MALT lymphoma, Conjunctivitis either alone or in the presence of a rheumatologic disease can increase the risk of conjunctival MALT lymphoma; Sjögren’s disease has a particular risk of pulmonary MALT lymphoma and then patients with rheumatoid arthritis are also at higher risk of MALT lymphoma.

So there is probably some people in the audience saying well MALT lymphoma doesn’t really happen in children, and that’s not entirely true although I’ll say MALT lymphoma is much more common in adults than in kids. But I happened to see a really interesting patient with Dr. Vanderlick and Dr. Terzic in clinic this past Monday and she is a patient who has an underlying immunodeficiency that looks like a partial De George syndrome. She has a chronic inflammatory skin disorder which is ill defined and we don’t understand. And she presented with what was
thought to be a pneumonia and it turned out to be a pulmonary MALT lymphoma. So a really interesting patient.

Back when I was at University of Michigan I’d seen two patients with tonsillar MALT lymphoma and these are kids who had been referred to their primary care physician because their tonsils are inflamed and they basically were being evaluated for tonsillectomy, and through the evaluation and pathology they in fact had MALT lymphoma of their tonsils. And then I’ve also seen a couple of patients who had salivary gland inflammation for no apparent reason and then they developed a MALT lymphoma; and a couple of patients with conjunctival inflammation with rheumatologic disease who developed MALT lymphoma. So this does occur in kids although it’s not certainly not nearly as common as Burkitt’s lymphoma.

So gastric MALT lymphoma is the most common form of MALT lymphoma and it is associated with a chronic H-pylori gastritis. This is just a pathologic section of the gastric lining and all the dark cells are the malignant lymphocytes infiltrating the submucosa. So this is kind of an over simplified generalized schema for the pathogenesis of MALT lymphoma, gastric MALT lymphoma. So there is an H-pylori infection that causes a chronic gastritis. You have a recruitment of lymphocytes to the area of chronic inflammation both T-cells and B-cells, and the T-cells provide a chronic stimulation to the B-cells causing them to proliferate. Along this process an abnormal clone emerges from these reactive B-cells within the site of chronic inflammation and there is also a contribution of specific cytogenetic abnormalities. And eventually a full blown MALT lymphoma emerges. Now like many
cancers MALT lymphoma is a spectrum of disease, there is patients with stage 1 disease, and for the most part those patients can be easily cured. There are patients with stage 4 disease who die of the disease. So one other thing I wanted to mention is that with the stage 1 disease patients 70% of the patients can be cured by treating them with antibiotics for H-pylori, and this reverses this process. So it’s very interesting. And what’s also interesting is asking the question what’s different about the other 30% who aren’t cured and we’ll talk about that in a little bit.

So two recurrent immunoglobulin heavy chain chromosome translocations occur in MALT lymphoma similar to the myc translocation and they are the 1:14 and I underlined the 14 to remind you that this is the immunoglobulin heavy chain, same thing that’s involved in the c-myc translocation in Burkitt’s lymphoma and a 14:18 translocation. This translocation happens in 10% of patients and the other in 20%, so together these represent 30% of gastric MALT lymphomas. The 1:14 translocation involves a gene that has the name BCL10, so when this translocation occurs what happens is that the BCL10 gene is translocated adjacent to the immunoglobulin heavy chain enhancer, and this leads to overexpression of this protein called BCL10. The 14:18 translocation involves a completely different gene also translocated adjacent to the immunoglobulin heavy chain promoter and the name of that gene is MALT1. So the 14:18 translocation results in MALT1 overexpression. So I just wanted to remind you that this is a recurring theme in B-cell non-Hodgkin’s lymphomas, genes that have important roles in regulating B-cell growth end up translocated adjacent to the immunoglobulin heavy chain, their gene transcription is deregulated and they become overexpressed.
So at the time these translocations were discovered which was about 1999 nothing was known about the proteins BCL10 and MALT1. And what I’ve shown here is just a domain structure of the proteins. So BCL10 if you enter its protein sequence into a program that can predict structure it has a domain near its aminoterminal end that’s called a CARD domain, it stands for a caspase recruitment domain which is a protein-protein interaction domain that’s present in lots of proteins that are involved in apoptosis and interact with caspases. MALT1’s domain structure is shown here, it has this thing at its aminoterminal it’s called a death domain in these two immunoglobulin-like domains and these are also protein-protein interaction domains. And then at its carboxy terminal end it has this domain called a caspase-like domain and it’s called this because the domain looks like the proteolytic domain that’s present in caspases that carry out their enzymes, that carryout cellular apoptosis. So it was believed when these two genes were described at these translocations that MALT1 may in fact be a protease but nothing was known about its function.

So my husband is Peter Lucas and he and I run our research lab together and when these translocations were discovered he asked a very simple question which basically led to the development of the rest of our research in our lab, and that is what is the relationship of BCL10 and MALT1? There are these two translocations, they never happened in the same patient but whether you have the first translocation involving BCL10 or the second translocation involving MALT1 you ended up with the same disease phenotype. So Peter reasoned that these two proteins probably have something to do with each other and when he gives talks he likes to say he made a – he did the dumb
man’s approach and he just put these two proteins into a cell and looked at what happened; and
amazingly enough it turned out that BCL10 and MALT1 bind to each other so you can measure this
in various ways including an immunoprecipitation assay that I’ll show you the results of. So the
answer is BCL10 and MALT1 bind to each other in the cytoplasm of B-cells and T-cells. So what
does that mean?

Well we further studied this interaction and what we found is that BCL10 when it bind to MALT1 it
actually pulls together two different MALT1 monomers, so it induces the oligomerization of
MALT1. And the way we studied this is that we engineered two different MALT1 monomers to
have different epitope tags that were recognized by different antibodies. So this MALT1 is tagged
with a little peptide called HA, this MALT1 is tagged with a peptide called MALT1 and we looked
at the interaction of these two different proteins either in the absence or in the presence of BCL10.
And we were able to demonstrate that when BCL10 was present these two things
immunoprecipitated with each other. In other words, the presence of BCL10 induced the interaction
of two different MALT1s. So that’s shown right here. On this top shell we are basically looking at
the ability of the HA tag MALT1 to interact with the myc tag MALT1 and it’s only when BCL10 is
present that we see this interaction.

So what happens when MALT1 is oligomerized? Well one of the things we thought might happen
was based on what we knew about EBV infection and B-cell lymphomagenesis, and we knew that
activation of NF kappa B transcription factor was absolutely essential to drive the proliferation of
malignant B-cells, so we wondered if BCL10 and MALT1 which presumably cause B-cell lymphoma because they are translocated in MALT lymphomas might also impact this very important signaling pathway. And so we asked the question could BCL10 and MALT1 activate NF kappa B? Does the oligomerization of MALT1 have some role in this process? And in order to do that we again genetically engineered a different type of MALT1. We replaced the part of MALT1 that binds to BCL10 with an artificial dimerization protein called FKBP. So here we have the MALT1 caspase-like proteolytic domain linked to this artificial dimerizing protein and when you add a drug and the drug’s name is AP1510, it’s just a complicated name, and these little circles are meant to be this drug. When you add the drug it causes the two FKBP’s to come together and the whole thing oligomerizes. So in fact we are artificially oligomerizing MALT1 and we are measuring what happens. And we are asking the question does that activate NF kappa B much like the EBV oncoproteins do? And this is the result of that, that experiment and this is what’s a luciferase promoter assay where we are looking at NF kappa B activation on the Y axis and we are looking at the concentration of this drug on the X axis and for our controls we looked at just the MALT1 part alone or the FKBP part alone and adding the drug didn’t do anything to NF kappa B, but for the fusion of the dimerizing area of protein with MALT1 we see a robust activation of NF kappa B. So this experiment was one of the many experiments that indicated to us that oligomerizing MALT1 induced the activation of this transcription factor. So maybe that’s how BCL10 and MALT1 contribute to lymphoma.
So since that time there has been a lot of labs working in this area and we’ve learned a lot. So we know that BCL10 and MALT1, two proteins whose genes were discovered because of their involvement in chromosome translocation in MALT lymphoma bind to each other in the cytoplasm of a lymphocyte. This blob is supposed to be a B-cell. When MALT1 is oligomerized we know that NF kappa B is activated and we know that NF kappa B can induce genes that cause the proliferation of the B-cell. The question we didn’t know is how is this whole process regulated? And that’s been studied by many labs over this past decade. And what we now know is that this complex is formed in response to stimulation of the B-cell receptor and it’s also formed in the T-cell in response to the T-cell receptor. So when the B-cell receptor is stimulated BCL10 and MALT1 come together in a complex next to the antigen receptor and this drives activation of NF kappa B. And this is actually required for normal immune function and if you generate a mouse that lacks BCL10 or lacks MALT1 those mice are immunodeficient and they can’t respond, their lymphocytes don’t proliferate in response to antigenic stimulation. So this makes sense – sorry – in terms of overexpressing BCL10 and MALT1 in the two chromosome translocations in MALT lymphoma. If you have too much of these things you can drive NF kappa B activation and cause the cell to proliferate in an inappropriate way causing lymphoma.

So there is a third translocation that happens in MALT lymphoma and that’s the 11:18 chromosome translocation and that’s a little bit different, it does not involve the immunoglobulin heavy chain but instead it causes the fusion of two coding regions. So you end up with a fusion protein that’s composed of a protein called API2 fused to a protein called MALT1, so this will encode the amino
terminus of the protein, attach to the carboxy terminus of MALT1. And the reason that I chose to study this 11:18 translocation is because as an oncologist I recognized that the phenotype of the MALT lymphomas with this translocation was a little bit different from the others. So the 11:18 translocation is seen in up to 40% of gastric MALT lymphomas and this translocation is associated with a failure to respond to H-pylori eradication and a development of this lymphoma in the absence of H-pylori. So it’s as if these tumors behave differently, they form in the absence of the chronic infectious process, they are also associated with larger tumor size with an advanced stage with a tendency to disseminate and several papers have shown that these tumors with this translocation are resistant to some of the currently available therapies for these patients. So I thought it would be interesting to ask the question how does this translocation contribute to this oncogenic process?

So here is the domain structure of MALT1 which we looked at before, has these protein-protein interaction domains on its aminoterminus and then a caspase-like domain closer to its carboxy terminus. MALT1 fuses with API2 which is also known as CIAP2 as a result of this chromosome translocation. API2 has on its aminoterminal end these three domains called BIR domains which are protein-protein interaction domains. And when the fusion protein is made this is what it looks like. And it always has the three BIR domains from API2 fused to the caspase-like proteolytic domain in MALT1 on its carboxy terminus. So we felt that since these domains were always retained in the fusion proteins of all the patients that these domains probably had an important role in contributing to B-cell lymphoma. And so we asked the question does API2 MALT1 fusion affect NF kappa B signaling? And we knew that MALT1 itself or API2 itself if you expressed those proteins in the cell
you wouldn’t see any activation of this NF kappa B transcription factor, but when you expressed the fusion protein we found that it was a profoundly strong activator event of kappa B and presumably this is how it contributes to the process of B-cell lymphomagenesis.

So the question we asked is why? Why does fusing this part of API2 to this part of MALT1 give this gain of function and cause it to be such a strong activator of NF kappa B? And one of the clues to figuring this out came from what we already knew from BCL10 and MALT1. We knew that BCL10 induced the oligomerization of MALT1 and that the oligomerization of MALT1 was required to cause NF kappa B to be activated. So we wondered if maybe the fusion protein API2-MALT1 might be already oligomerized. And if it’s already oligomerized that would mean that as soon as it’s expressed in the cell you would have strong activation of NF kappa B and we know that’s important to the process of B-cell lymphomagenesis. And the reason that we ask this question has to do with my pediatric hem/onc fellowship in that I had seen a lot of patients with some very interesting chromosomal translocations and I had learned that this notion of inappropriate oligomerization of fusion oncoproteins is also a recurring theme.

And there are many examples in leukemia and lymphoma. So the most famous example is the Bcr abl fusion protein that’s formed by the Philadelphia chromosome in CML and some cases of infant – of high risk ALL. And abl is a tyrosine kinase and when it is activated it becomes a dimer. And when it’s dimerized it can drive certain signalling pathways. When abl gets fused to this protein called Bcr because of a chromosome translocation it causes abl to be dimerized constitutively or in
an unregulated way. And this is actually what drives and cause CML. Similarly there is a fusion protein that happens in anaplastic large cell lymphoma where there is an anaplastic large cell kinase that under normal circumstances should exist as a monomer, but when it’s fused to NPM because of this – of a chromosome translocation creating a fusion oncprotein this becomes constitutively oligomerized and the ALK tyrosine kinase is turned on and ALK, the tyrosine kinase activity drives the development of this cancer. There is other examples too including the PML RAR fusion oncprotein in promyelocytic leukemia and also the MLL fusion proteins in infant leukemia. So there is examples in the literature of fusion oncproteins having their oncogenic property because they oligomerize when they are really not supposed to and I wondered if the same thing happened with API2-MALT1, could it be that it’s always an oligomer and that’s why it causes lymphoma?

And it turns out that we were able to prove this, and I won’t show you much data. Suffice it to say that we did an immunoprecipitation study again where we looked at the ability of two different API2- MALT1s to interact and at baseline they are constantly oligomerized. And then if we looked at which part of this protein mediates this oligomerization we were able to prove that it was the API2 portion of this fusion oncprotein that was responsible for causing this to come together as an oligomer. So this helped us to understand why API2-MALT 1 as an oncprotein was so powerful and why it didn’t require any H-pylori in gastric MALT lymphoma. So BLC10 and MALT1 come together in response to B-cell receptor signaling, but API2-MALT1 is constitutively oligomerized so it can bypass the normal regulation of the antigen receptor and drive NF kappa B activation and drive lymphomagenesis. So now we have these 3 examples of chromosome translocations, the 1:14
translocation that over expresses BCL10; the 14:18 translocation that over expresses MALT1 and the 11:18 translocation that causes this fusion protein. Each of these would be expected to drive activation of this NF kappa B transcription factor and contribute to the process of B-cell lymphoma.

So something really exciting that’s happened in the last 5 or so years is that Lou Staudt’s group at the National Cancer Institute, he runs the Lymphoma Branch of the NCI, has been sequencing large numbers of lymphoma cases and identifying new mutations in lymphomas. And one thing they found is they found 10% of patients with a certain subtype of diffuse large B-cell lymphoma have activating mutations in a protein called Carma1. Carma1 is a scaffolding protein that’s composed of multiple protein-protein interaction domains. It interacts with BCL10 and brings the BCL10-MALT1 complex up to the B-cell receptor when the B-cell receptor is stimulated. In these patients with this mutation the mutation causes Carma1 to unfold in such a way that it constantly interacts with BCL10 thereby constantly driving this pathway leading to the development of lymphoma. So now this pathway that was discovered because of its involvement in MALT lymphoma turns out to have an important role in the pathogenesis of diffuse large B-cell lymphoma as well. Of course I was super excited about that because diffuse large B-cell lymphoma is much more common in pediatrics, we see tons of patients with this disease.

In a second paper by Lou Staudt’s group at the NCI they found 20% of patients had a mutation in a B-cell receptor subunit called the CD79b subunit. This mutation causes this pathway to be
constitutively activated and causes the B-cell receptor to constantly recruit Carma1 and therefore BCL10 and MALT1 to its receptor. So now we have 30% of diffuse large B-cell lymphoma patients having mutations that drive this pathway and therefore cause lymphoma.

The other reason that I think this is exciting is to look back about EBV and to realize that there is significant mechanistic overlap between MALT lymphoma, diffuse large B-cell lymphoma and EBV driven lymphoma in that all of these lymphomas are driven by inappropriate NK kappa B activation which changes the gene transcription profile of the B-cells and causes them to take on malignant properties. And finally I just want to add that figuring this out and you know we have contributed to this process, it has been extremely rewarding because it has helped us see how new drugs might become available for patients with lymphomas. And I’ll give you some examples.

So there are tyrosine kinases including the tyrosine kinase Syk and the tyrosine kinase Bruton tyrosine kinase which are known to be intermediaries in the activation of Carma1 and the pulling together of this signalling complex; and there are inhibitors for these tyrosine kinases that are now in clinical trials and there is recently a very exciting trial with a new Bruton tyrosine kinase inhibitor in a subset of refractory diffuse large B-cell lymphoma patients. And the results were very exciting that as a single agent this could induce remission in some of the patients that had refractory disease with mutations in the CD79b subunit.
Perhaps most exciting to us in our lab is the development, the potential development of MALT1 inhibitors. So in the last 2 years MALT1 has been crystalized and it turns out that like we had predicted MALT1 exists as a dimer. And in fact it does have proteolytic activity, its proteolytic activity may have an important role in this process and there are many drug companies involved – excited about developing MALT1 inhibitors as potential agents for blocking this process and thereby treating patients with refractory lymphoma. And in fact there are some preclinical studies recently published with some novel inhibitors that were just discovered and they showed the ability to prevent the growth of diffuse large B-cell lymphoma cells in culture, so that’s very exciting.

And then finally there is a class of drugs that inhibit NF kappa B and those drugs are already incorporated into the Children’s Oncology Group Studies including Bortezomib. So I hope that I’ve convinced you that doing these scientific investigations has really been fruitful in the world of B-cell non-Hodgkin’s lymphoma and offers our patients potential new strategies.

So just some take-home points on MALT lymphoma. MALT lymphoma occurs at sites of chronic inflammation, gastric MALT lymphoma is the most common form and it occurs in association with H-pylori infection. The immunoglobulin locus translocations result in overexpression of BCL10 and MALT1. The third translocation, the 11:18 creates an API2-MALT1 fusion oncoprotein. All three of these translocations drive the inappropriate activity of the NF kappa B transcription factor. This same proinflammatory NF kappa B signaling pathway is now implicated in diffuse large B-cell lymphoma and there is signification mechanistic overlap between MALT lymphoma and EBV
driven lymphomas which also depend on inappropriate activation of the NF kappa B transcription factor. And all of these molecular insights have provided the impetus for developing new therapeutics for our patients. So the take-home message is that many cancers involve a complex interplay between infection, inflammation and cancer including B-cell non-Hodgkin’s lymphoma.

And I’ll just end by saying thank you to all of the members of our lab and I’ll point out that Peter Kuffa is the person who did the API2-MALT1 oligomerization studies. And then I’ll also say thank you for coming today and tell you that I’m super, super excited to be here. We are so excited to be starting our lab in the Rangos Building, I’m so excited to be working with the Pediatric Hem/Onc Division, and if there are any medical students here or residents who think this maybe piqued an interest either in our lab or in pediatric hem/onc as a specialty I want you to please come talk to me. Okay? All right, thanks.