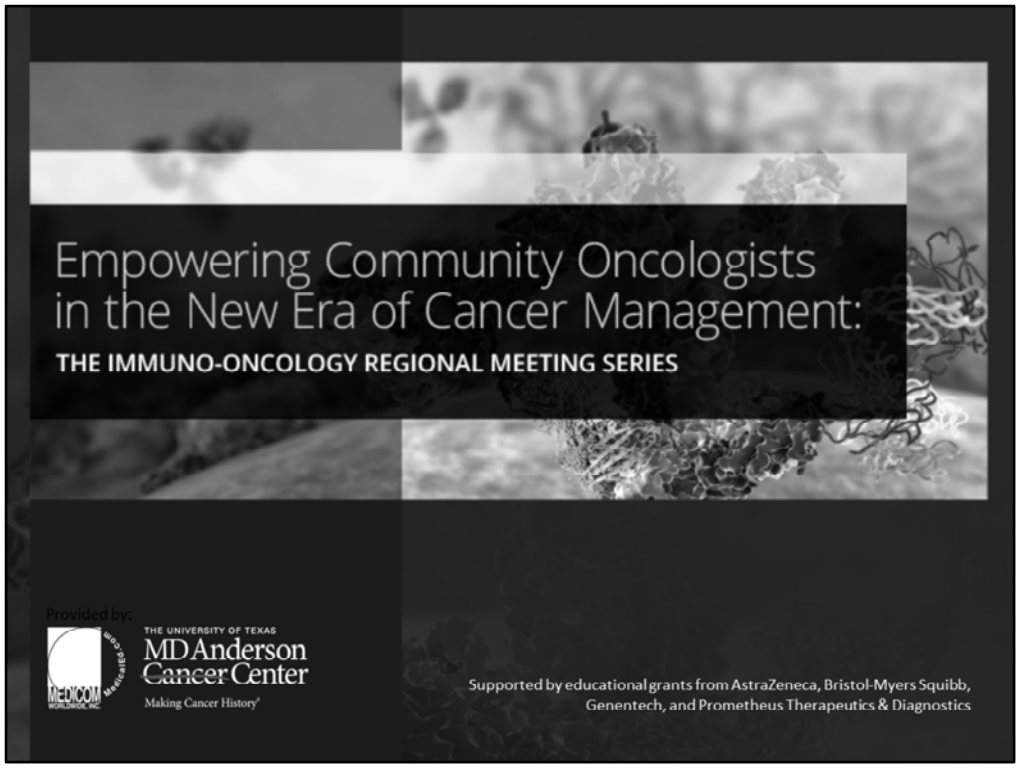
Module 1



We are hopeful that we can teach you some useful background and tips about using immunotherapeutics for the treatment of cancer. I am Jason Luke from the University of Chicago where I do melanoma and solid tumor phase one with a focus on immunotherapy. Today, for the rest of the panel, we have from the Moffitt Cancer Center, Dr. Jeffrey Weber, and from Emory University Dr. Carthon. They are going to be speaking a little bit later,

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The Principles of Tumor Immunology and Immunotherapy: Rationale for Immunotherapy

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Okay, and with the first talk today, I am going to go over principles of tumor immunology and immunotherapy and the rationale for immunotherapy. We are going to find that this talk may be quite basic for some people, and they may know all of this material already. Other people in the audience really may have had very limited exposure to this material. So, if it is going too fast, follow up with us afterwards or raise your hand, I guess. If it is too little, then sort of bear with us.

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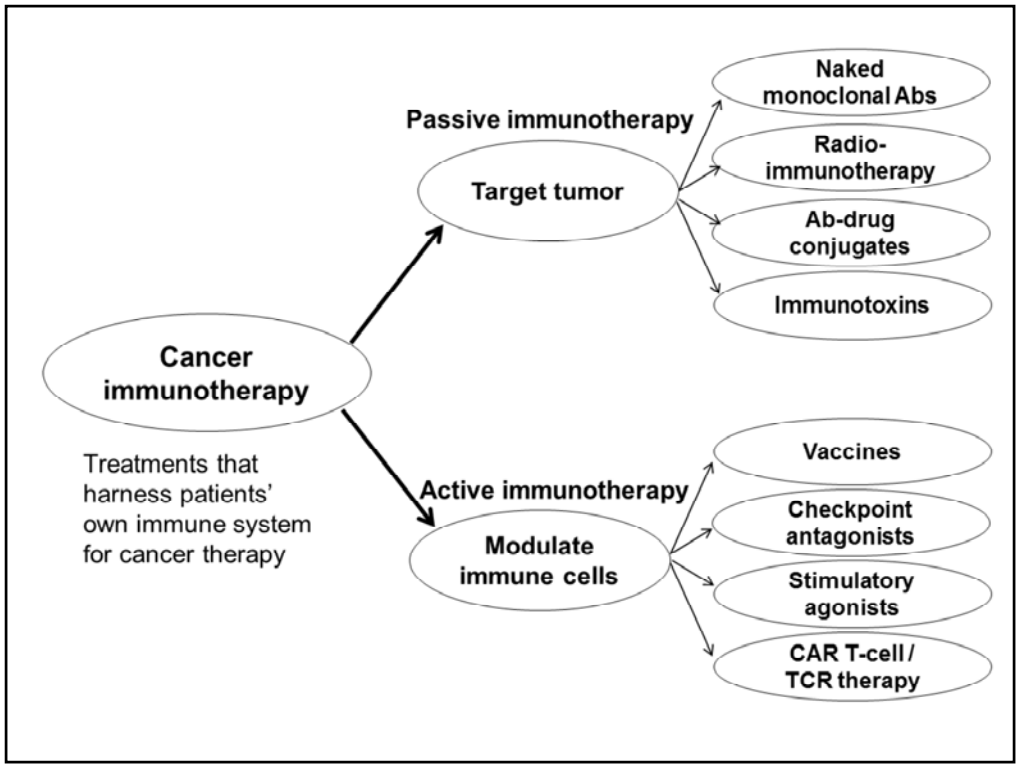

So, conceptually speaking throughout this talk and throughout the day, we are going to discuss cancer immunotherapy in terms of passive immunotherapy and active immunotherapy. So, cancer immunotherapy in general are treatments that harness the patient's own immune system to fight cancer. Active immunotherapies are drugs that we give to try to modulate the immune response in hopes of inducing an antitumor immune activity. Passive immunotherapy can be split into different categories. We are going to come back to this near the end to go over this again, but these can include monoclonal antibodies, naked monoclonal antibodies that basically bind to a tumor or tumor antigen, radioimmunotherapy where radiation is linked to an immunotherapy approach, antibody drug conjugates, this is something like TDM-1 where an antibody can stick to a target and then bring in a drug, whether it be chemotherapy or other, or immunotoxins which can similarly bind to elements within the immune system and bring in other therapies. Active immunotherapy includes vaccines which can induce immune response, checkpoint agonists or antagonists, and we will discuss those at length at the end of my talk and Dr. Weber's talk, as well as chimeric antigen receptor T cells or T-cell receptor based therapy, and those may be new to some folks in the audience, and again, we will go over them a little bit later in the talk.

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Passive vs Active Immunotherapy

Passive immunotherapy (mAbs)	Active immunotherapy	Benefit of active immunotherapy
Passive immunotherapy	Active immunotherapy	B and T cell response
Monoclonal	Polyclonal	Minimizes immune escape
Lasts for few months	Lasts lifelong due to immunological memory	Lifelong benefit
Targets surface molecule	Targets surface or intracellular molecule	Complementary to passive immunotherapy

So, the first approximation in understanding the immune system, it is important to differentiate the innate versus the adaptive immune components to the immune system. So, starting from the left, the innate immunity is really the basic immunity that all multicellular organisms have, not all, but most larger multicellular organisms have. It includes cell lines such as neutrophils, eosinophils, basal cells, and monocytes, which are really the first response to an infection, and these are the ones we are commonly understanding in clinical medicine when we evaluate for bacterial infections, and this portion of the immune response has no specific memory. It is a response to an initial infection, and it is not specific particularly to that infection. That is contrasted with adaptive immunity which you see on the left-hand side, your right-hand side, which includes B cells and T cells which develop in response to a specific infection, so the response is specific, and it can induce an immunological memory such that if the immune system is challenged a second time, that response can ramp up quickly once again, and the innate and adaptive immunities are linked by antigen-presenting cells which help to take the nonspecific approach and convert it into a specific approach, and we predominantly think of that as being modulated by dendritic cells, the major antigen-presenting cell. So, again, components of passive versus active immunotherapy in the context of innate and adaptive immunity, passive immunotherapy is generally monoclonal, meaning it is a response to some sort of infection as opposed to active immunotherapy which can sort of be developed against many different sort of challenges. Passive immunotherapy, generally speaking, lasts for a shorter period of time because it is a direct challenge to the tumor or whatever the infection is, whereas active immunotherapy once it develops can have a long-lasting memory. Generally speaking, with passive immunotherapy, when we think about monoclonal antibodies, the target has to be on the surface of the cell. It has to stick to the cell in order to have its function, whereas with active therapies this can be more nuanced. It can be surface targets or it can be intracellular proteins or other molecules, and the benefit from this is that active immunotherapy as compared to passive can minimize escape. So, as you can develop resistance to a passive immunotherapy, with active immunotherapy at least theoretically, the benefit potentially can be long lasting, and it can modulate to encompass any resistance. The benefit can be lifelong, and potentially active immunotherapy can be complementary to passive immunotherapy, we will discuss how a little bit later on.

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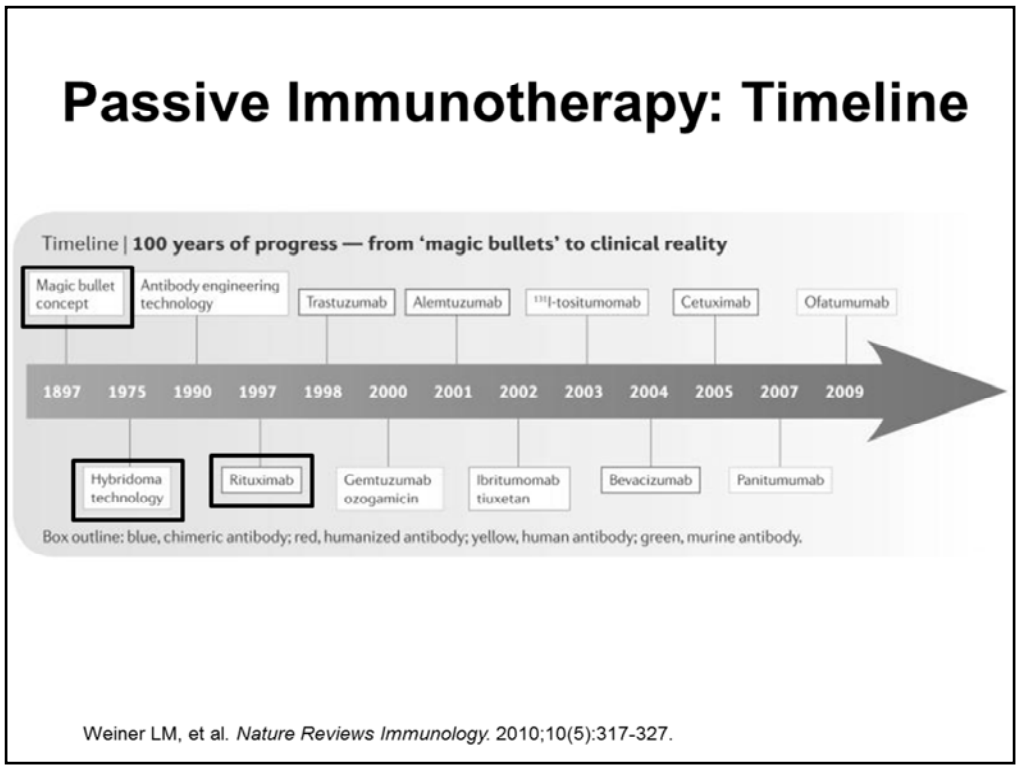

So, in thinking about a timeline of how the immune system and how immunotherapies have been developed, we will take them in two flavors, the first being passive immunotherapy, and if you can see on the left-hand side of this, this goes back quite a ways, and so if we think back to the late 1900s, 1897, Paul Ehrlich proposed a magic bullet concept which really is a foundational principle really across oncology at this point. The magic bullet concept is essentially the lock-and-key receptor ligand interaction, and though he obviously did not understand exactly the specifics of how it would play out, that was an important thought process and has really guided how he developed even target therapies now in the 21st Century. If we fast forward through some of this, it was in the 1970s that we developed hybridoma technology, and Milstein and Köhler developed and won the Nobel Prize for this technology, and essentially, the short version of this is if you take a B cell which makes antibodies and you fuse it with a myeloma cell which is transformed, it can live forever, do some other machinations and essentially have a cell that can make the same kind of antibody for an open-ended period of time. Therefore, if you design an antibody or if you develop an antibody, you can continue to make it, and that was very important in essentially developing clinical grade reagents. The first of these passive immunotherapies to develop was rituximab with the anti-CD20 antibody which is used to cross hematologic malignancies, and since then, there have been a host of other immunotherapies developed you can see on the far right, ofatumumab which is another anti-CD20 antibody. We will contrast those here a little bit but others such as cetuximab anti-EGFR antibody.

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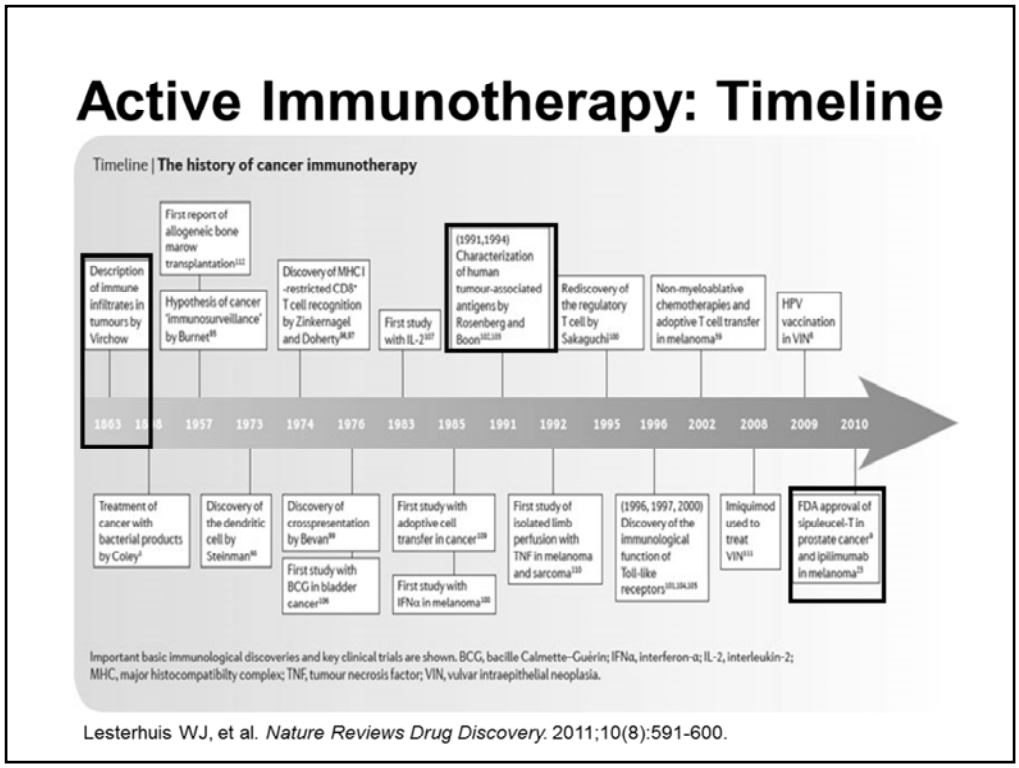

Lesterhuis WJ, et al. *Nature Reviews Drug Discovery.* 2011;10(8):591-600.

With active immunotherapy, the timeline is similarly long ago. If we go back here to 1863 and think about the description of the first immune infiltrates within a tumor by Virchow, that has started a field which only recently has really come into the clinical practice. The development of active immunotherapies was sort of highlighted in the 1990s by the characterization of tumor-associated antigens which was a foundational principle for the development of guiding the immune response toward a specific antigen but culminated in 2010 with the approval of sipuleucel-T for prostate cancer and in 2011 for ipilimumab in melanoma, and we will discuss those at length here today.

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FDA Approved Active Cancer Immunotherapies

- 2010: Sipuleucel-T (PROVENGE - dendritic cell vaccine) for prostate cancer
- 2011: Ipilimumab (Yervoy - anti-CTLA-4 Ab) for advanced melanoma
- 2014: Pembrolizumab (Keytruda - anti-PD-1 Ab) for advanced melanoma

So, there are three active cancer immunotherapies that are approved right now: 2010, sipuleucel-T or Provenge, the dendritic cell vaccine for prostate cancer; 2011, ipilimumab or Yervoy, anti-CTLA-4 monoclonal antibody for melanoma; and then this year, pembrolizumab or Keytruda, anti-PD-1 antibody again for melanoma. This, you can see, is more recent, has really developed a groundswell as the basis for this because there are a whole host of modulatory immune responses which are rushing into the clinic, essentially across all cancer histologies, which are going to become foundational principles in the treatment of cancer moving forward,

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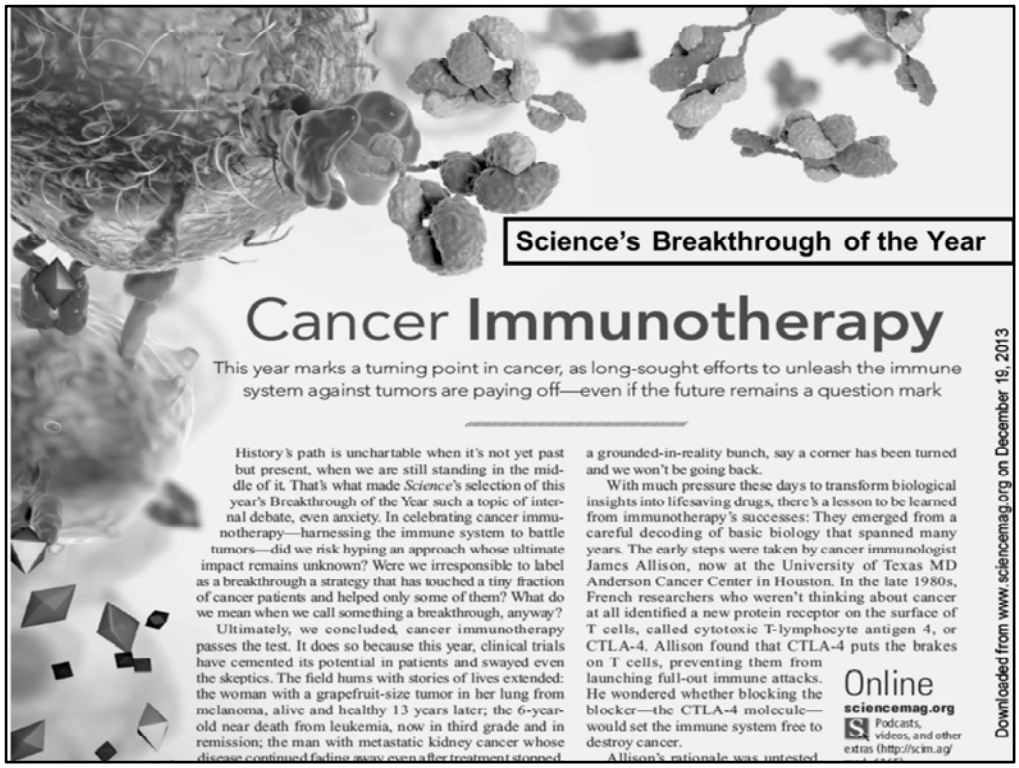

Science's Breakthrough of the Year

Cancer Immunotherapy

This year marks a turning point in cancer, as long-sought efforts to unleash the immune system against tumors are paying off—even if the future remains a question mark

History's path is unchartable when it's not yet past but present, when we are still standing in the middle of it. That's what made *Science*'s selection of this year's Breakthrough of the Year such a topic of internal debate, even anxiety. In celebrating cancer immunotherapy—harnessing the immune system to battle tumors—did we risk hyping an approach whose ultimate impact remains unknown? Were we irresponsible to label as a breakthrough a strategy that has touched a tiny fraction of cancer patients and helped only some of them? What do we mean when we call something a breakthrough, anyway?

Ultimately, we concluded, cancer immunotherapy passes the test. It does so because this year, clinical trials have cemented its potential in patients and swayed even the skeptics. The field hums with stories of lives extended: the woman with a grapefruit-size tumor in her lung from melanoma, alive and healthy 13 years later; the 6-year-old near death from leukemia, now in third grade and in remission; the man with metastatic kidney cancer whose disease continued fading away even after treatment stopped,

a grounded-in-reality bunch, say a corner has been turned and we won't be going back.

With much pressure these days to transform biological insights into lifesaving drugs, there's a lesson to be learned from immunotherapy's successes: They emerged from a careful decoding of basic biology that spanned many years. The early steps were taken by cancer immunologist James Allison, now at the University of Texas MD Anderson Cancer Center in Houston. In the late 1980s, French researchers who weren't thinking about cancer at all identified a new protein receptor on the surface of T cells, called cytotoxic T-lymphocyte antigen 4, or CTLA-4. Allison found that CTLA-4 puts the brakes on T cells, preventing them from launching full-out immune attacks. He wondered whether blocking the blocker—the CTLA-4 molecule—would set the immune system free to destroy cancer.

Allison's rationale was untested,

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so much so that last year the *Science* magazine actually described cancer immunotherapy as the breakthrough in science of the year. It is really the foundation now for how we are starting to think about next-generation approaches for cancer, whether it be combinations of immunotherapy with chemotherapy, targeted therapies, and other ways of thinking of treatment of cancer.

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Brief Primer on Immunology

So, with that background, we are going to have a very brief primer on immunology to give you some foundational principles about how to think through why some of the effects of immunotherapy take place and how to sort of think of them in advance.

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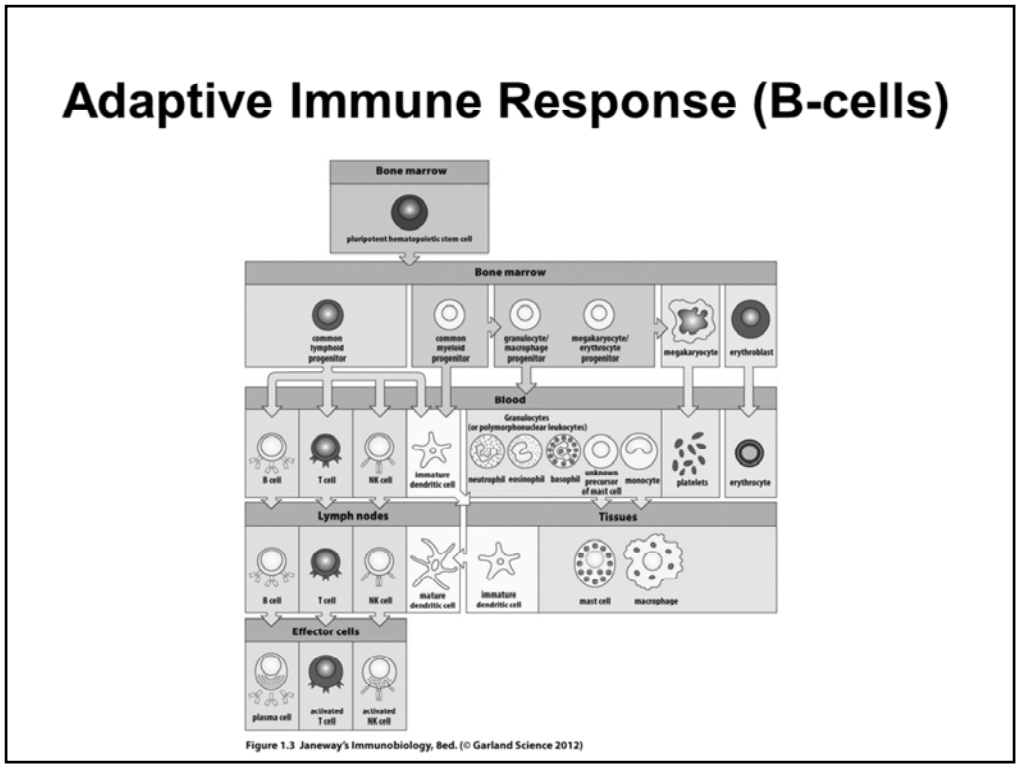

I am going back to the adaptive immune response to talk through how these cells develop is very important in understanding their functions, and so if we go back to the bone marrow to a pluripotent hematopoietic stem cell, that cell can then differentiate into a host of different immune cells. Overall, I remember this from medical school histology class. On the right-hand side, you can see myeloid series of cell lines eventually differentiating into granulocytes as well as mega karyocytes and red blood cells. Of interest to active cancer immunotherapy however, it is going to be the left-hand column where we have differentiate from a pluripotent cell into a common lymphoid progenitor and eventually into naïve B, T, and K cells.

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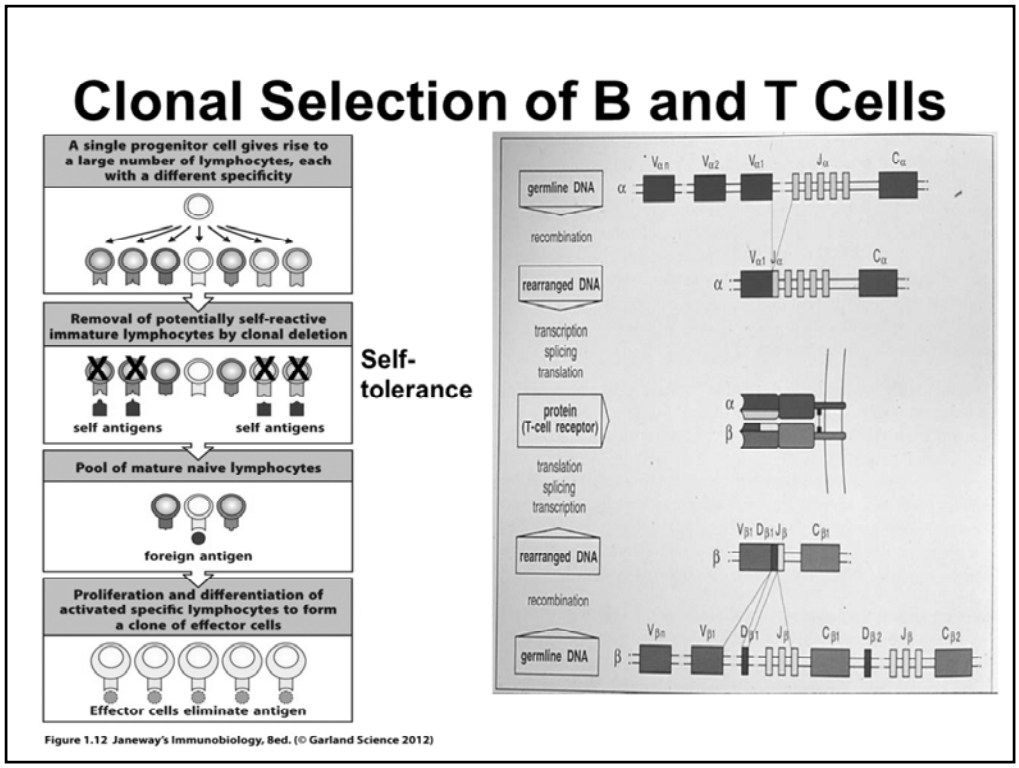

The clonal selection of B and T cells is very important when understanding the specificity of the immune system, so if we think back to how an immune cell can become specific, initially on the upper left-hand side we have single progenitor cell that give rise to a number of lymphocytes. These all have a different specificity, some potentially to self-antigens which potentially would be an issue. Those then various mechanisms exposed to self-antigens, and the cells that are reactive against the host against our own bodies are then deleted through a processes known as self-tolerance. That pool of mature naïve lymphocytes then can then be available throughout the body to be exposed to foreign antigens to generate a very specific immune response, and after that takes place, you are going to have have proliferation and differentiation of these, which are now very specific to that antigen that was exposed. And how does that specificity, how is it developed, and to take for example here, we look at the T cell receptor which is specific to very particular antigen. If we look at the alpha chain, how this specificity takes place is that within the DNA sequence of the T cell there are segments known as the V, J, and C regions which can be recombined into specific DNA fragments, and that can be translated into a protein which has a very specific specificity. Similarly, the beta chain has segments known as V, D, and J, which again can all be put together, and now you have two fragments or two parts of the T cell receptor which each have a very high specificity for a certain antigen, which they can then find should they be exposed to it.

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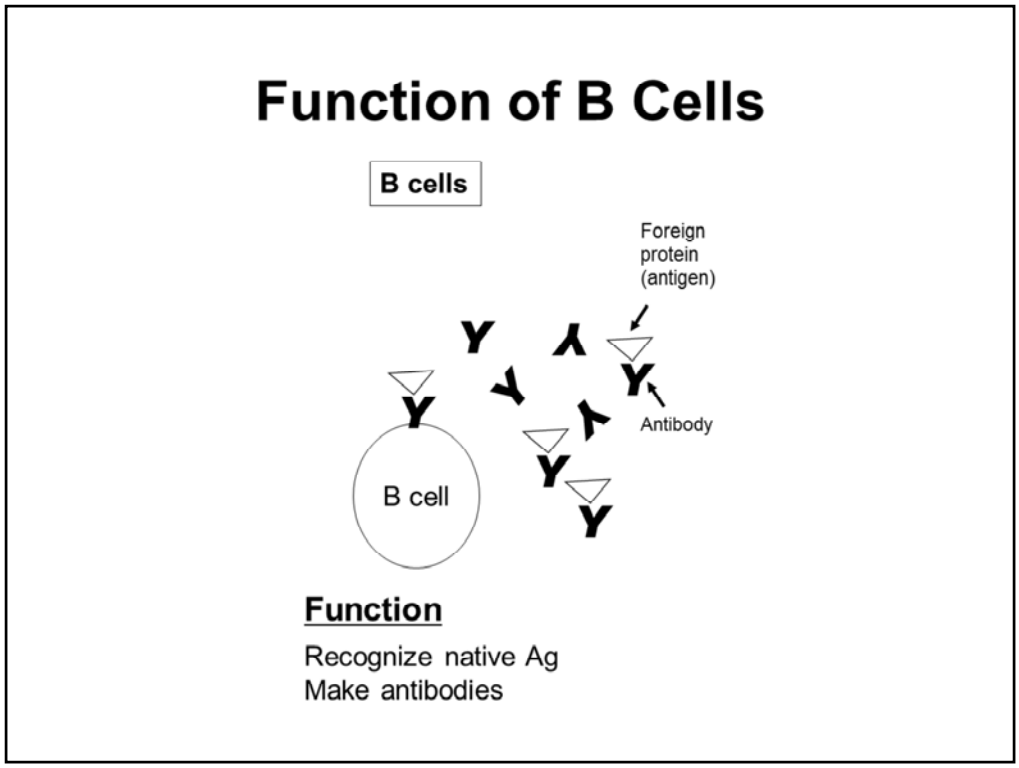

So, the function of B cells which are one part of adaptive immunity are to develop antibodies, and antibodies are immunoglobulin proteins that circulate and bind to their cognate antigen. B cells can recognize large antigens or naïve antigens that have not yet been processed, so things floating around in your body once they stick they can have activities in generating other immune factors to do their jobs. They can also be shown on the surface of a B cell, and if that antigen comes in contact, it then activates that B cell to make even more antibodies.

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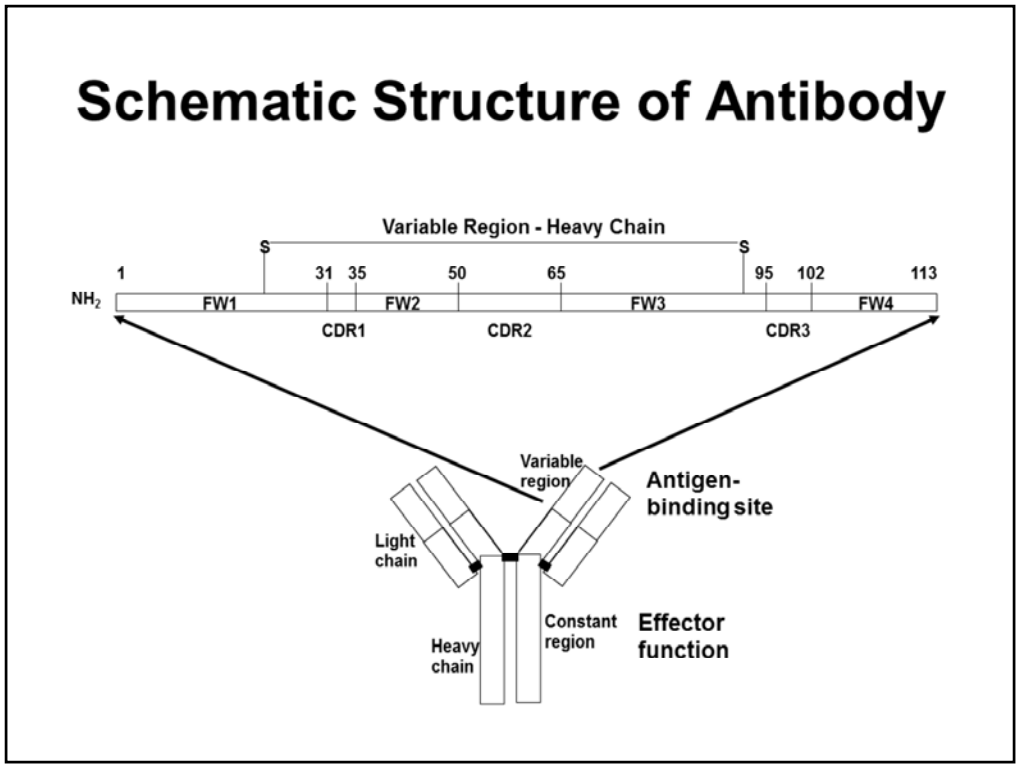

What is an antibody? An antibody has different components or different parts that are important to understand at least conceptually. When I was memorizing this back in college, I remember thinking how am I going to remember this? The easy way to think about this is that there are two parts, the Fab and the Fc. So, if you just look at it, you can sort of see it. The top is the Fab, and the bottom is the Fc, and they are made up of different parts. On the left here, you can see that this fragment is known as the light chain, and the central portion is known as the heavy chain. The binding aspects of the antibody take place on the edges out here, which are made up of the variable regions as opposed to the bottom which is the constant region which has effector functions, and how is that variability region or the binding specificity determined, and that takes place through elements of what I just referred to for the VDJ recombination. So, the light chain has constant and variable regions, and within that variable region, they have recombination of what I have referred to earlier, and within the heavy chain, we have recombination within these three sequences in orange, the CBR1, complementary determining regions one through three. And so you can remember there is those different DNA sequence that are getting put together, and if you do that in three different places and combine it with the one within the light chain, you now have a high specificity for one specific antigen.

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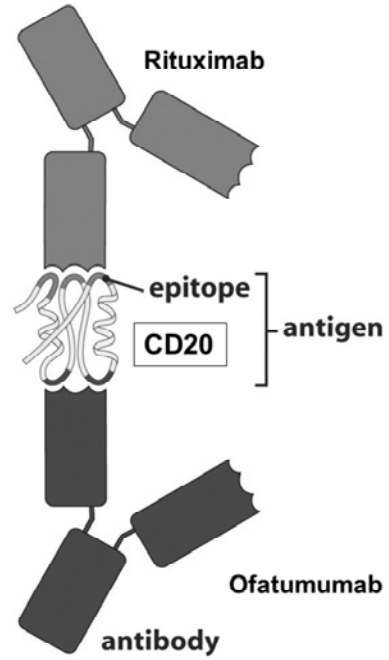

Figure 1.15 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

So, when we think about how is this relevant to clinical practice? If we go back to the molecules that we are aware of, so again on the top, we see rituximab. It is a monoclonal antibody that has specificity within the binding domain to a particular epitope of CD20. Okay, and we know that that works. The drug is part of the standard armamentarium for the treatment of hematologic malignancies. Ofatumumab is another anti-CD20 antibody. That antibody similarly binds to CD20, but you can see here binds to a different part of CD20, and this goes to the specificity within the protein of what exactly that it binds to. So, this is an important concept when thinking about molecular antibody therapy, you may hear, "Well, wait a minute, with all these drugs, they are all being developed against the same thing," but they are not exactly the same drug because they bind to different things, and theoretically, they could have very different activities in patients, and that is why we still need to do the clinical trials to determine what their utility is. I guess I will just say it is sort of likely obvious to those, but an antibody that binds to a molecule, that molecule or the part that it binds to is known as antigen, and so when you hear that lingo, antibody-antigen binding, this lays out sort of what we are referring to.

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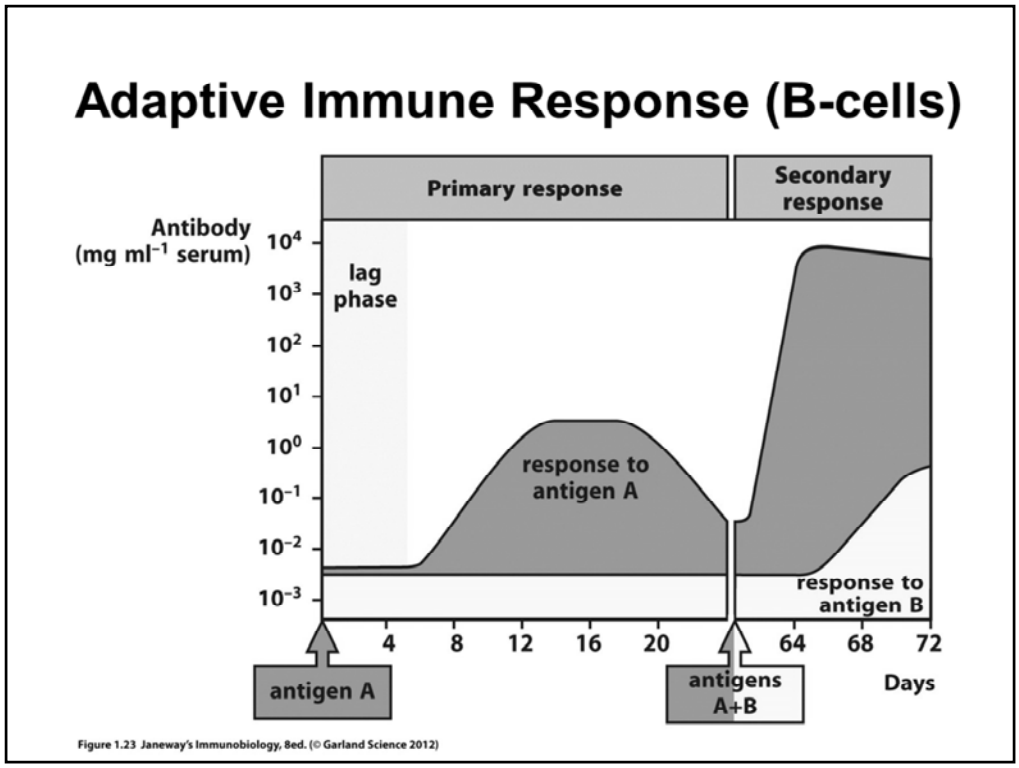

It is when we think about the B cell adaptive immune response, what we can see is that when the immune system is challenged with an antigen or a protein to amount of response, there is a little bit of lag phase because the immune cell has to be stimulated, has to grow, start to secrete, you eventually, however, get a response to that antigen. That response once the infection is cleared then goes down, but after a rechallenge, you can see that you get a much more robust and rapid response the second time. So, here we have initial exposure, a little bit of a lag phase, and then response. This then goes down, but you have a rapid response once you are challenged the second time, but you can see that if you are challenged with the second antigen, this is antigen A and this is antigen B, that takes longer to develop similar to the first time. So, that is the function of B cells again to make antibodies which bind to things in the extracellular environment.

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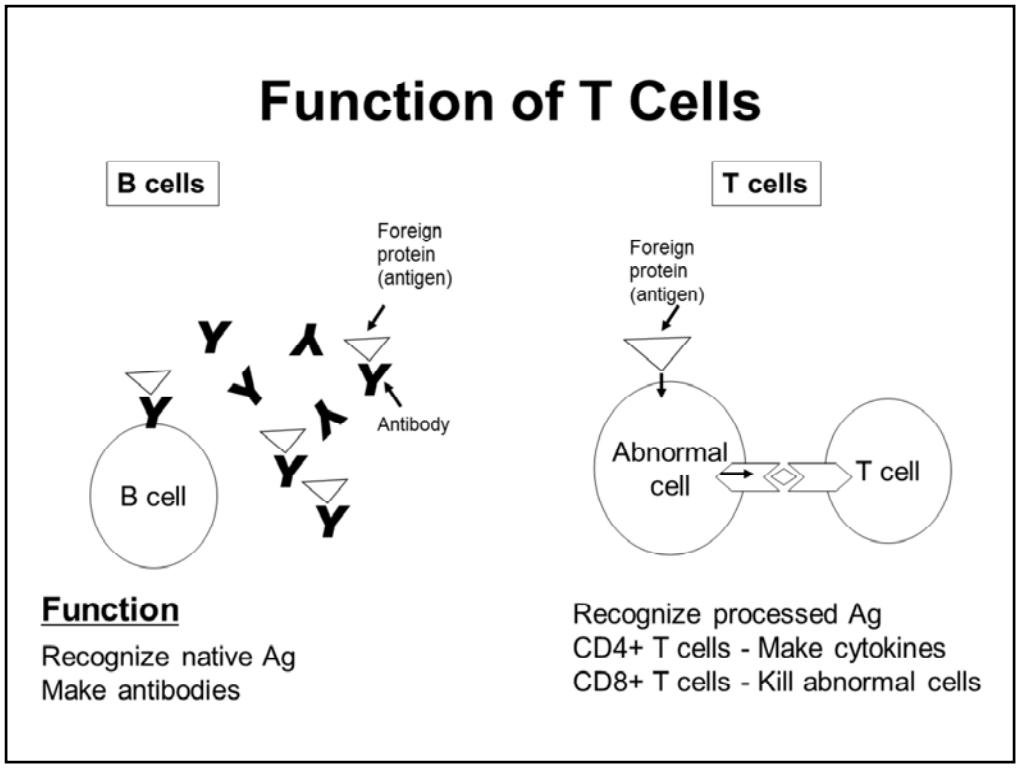

T cells are the other half of adaptive immunity, and what these cell do are the effector functions within the immune system, and commonly, we think of them in two flavors, CD4 and CD8 positive T cells, and what these cells do is that after they recognize their specific antigen, in the case of CD4 cells, they commonly makes cytokines which can boost the activity of other immune cells. CD8 T cells then can go and actually kill other cells that are expressing abnormal molecules. So, here we have an abnormal cell, call it a tumor cell, that is expressing a foreign protein like a tumor protein. When it comes in contact with the T cell, that T cell then potentially could kill the tumor cell if that T cell knows to look for it.

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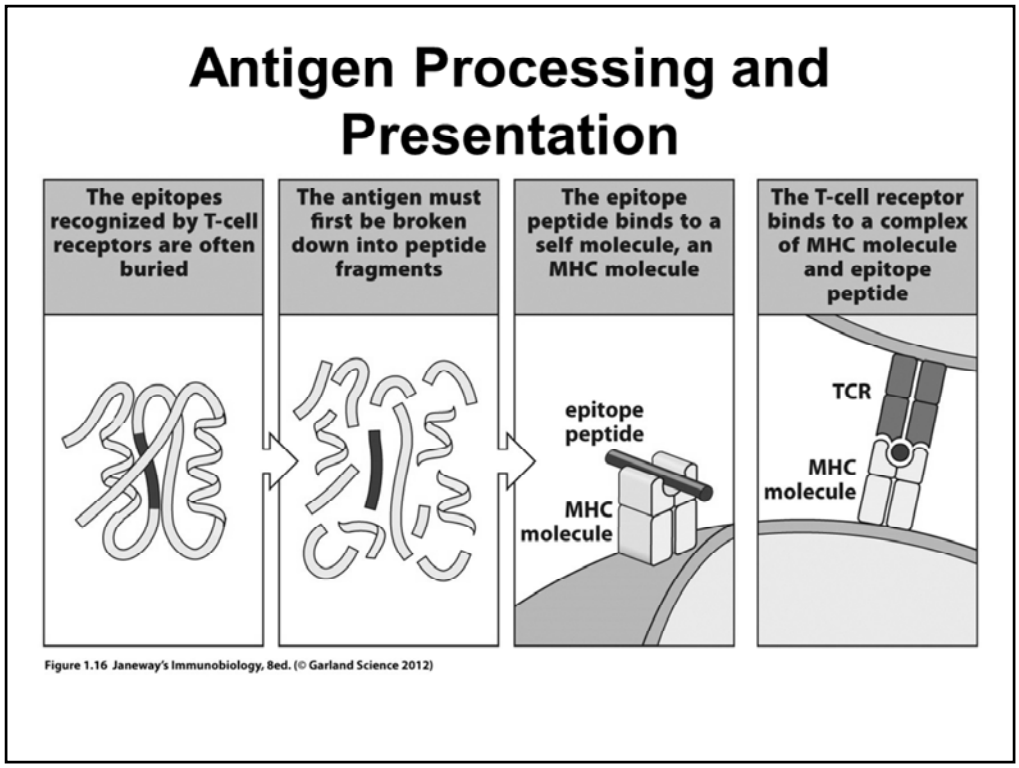

So, how is that specificity in the T cell developed? Again, we talked about antigens and how antibodies can bind to things in the extracellular environment such as the epitopes on antigens. So, we saw for example of CD20, we had antibody that was binding here. For T cells, the peptides or the antigens that they bind to are much smaller such that what needs to happen for them to notice these is that a large antigen such as this needs to be processed. So, the antigen has to be broken down in the peptide fragments. Those fragments then need to be processed into a vehicle to be shown to the T cells in order to stimulate them. That vehicle is known as MHC or HLA in humans, and after an antigen is processed, each peptide epitope can be put into an MHC, then expressed on the surface of the cell, and shown to the T cell receptor of T cell.

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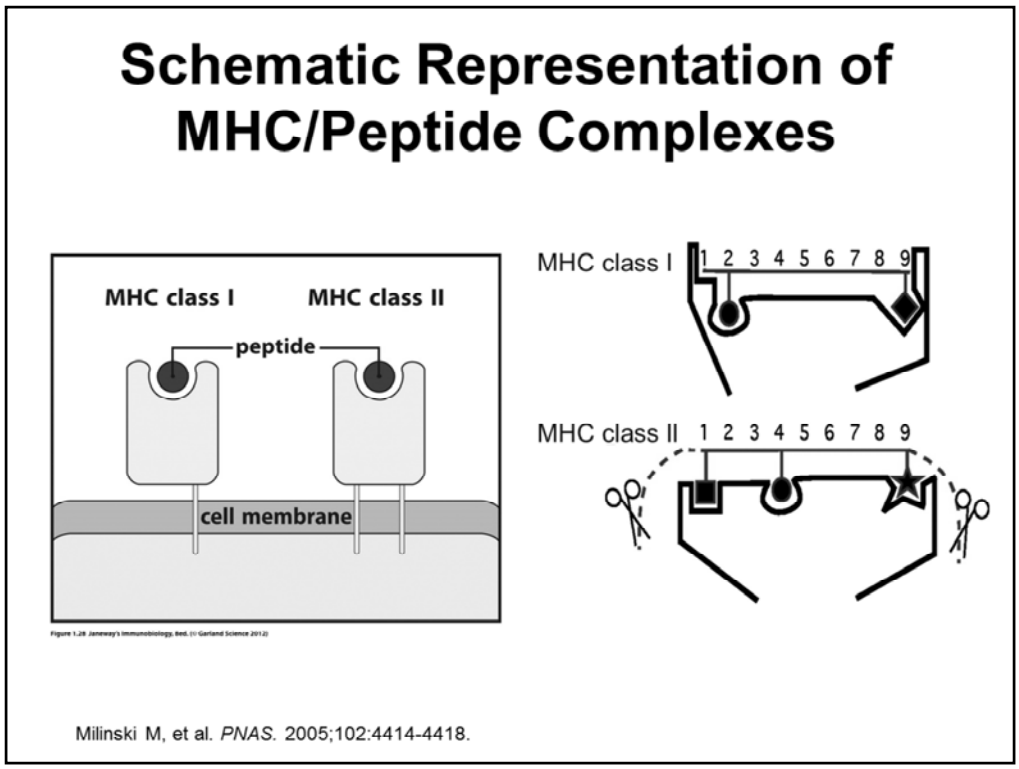

So, understanding MHC class I versus class II is an important concept when we think about cancer immunotherapy. MHC class I proteins display proteins that are shown on the inside of the cell, so almost all cells in your body express MHC class I, and essentially, what this does is it sort of regurgitates in some sense the molecules that are within the cell to the surface of the cell to show them to the immune system such that if a normal cell in your body is infected with a virus, some of those virus particles will eventually get shown on the surface of the cell. The T cell then can come and read all of the MHC, and if they see these viral particles, the T cell will then kill that cell so the virus does not continue to replicate. Class II MHC expresses peptide, generally speaking, that are in the extracellular environment, so infections that take place in your blood stream per se that they do not go inside of cells, they still release particles or antigens which can then be processed and displayed on MHC class II. Those can then stimulate the CD4 cells which can then release cytokines and try to increase the overall immune response, and the binding of class I and class II peptides is slightly different. Again, these are processed from inside. These, generally speaking, are antigens from inside the cell. The binding affinity for MHC class I is, generally speaking, limited to a sequence of amino acids of approximately 9 in length whereas with MHC class II this can be longer, again sort of owning to what we are looking for. We are looking for very specific small molecules within the cell as opposed to extracellular particles.

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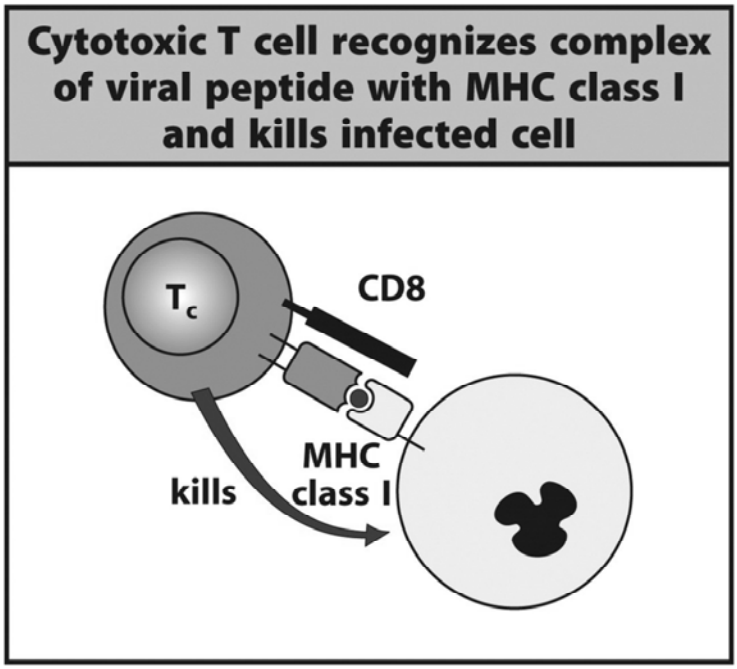

Figure 1.30 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

So, then the function of these cells, so CD8 cells again we think of as effector cells or sometimes killer of T cells, so this is a cytotoxic T cell, has expression of CD8, and what CD8 does is that after the T cell receptor finds an MHC class I that has a peptide that it is aware of, the CD8 helps to bind to the MHC and that can trigger molecules secreted from the cytotoxic T cell, which can then kill that cell, whether it be a tumor or other bad-acting cell.

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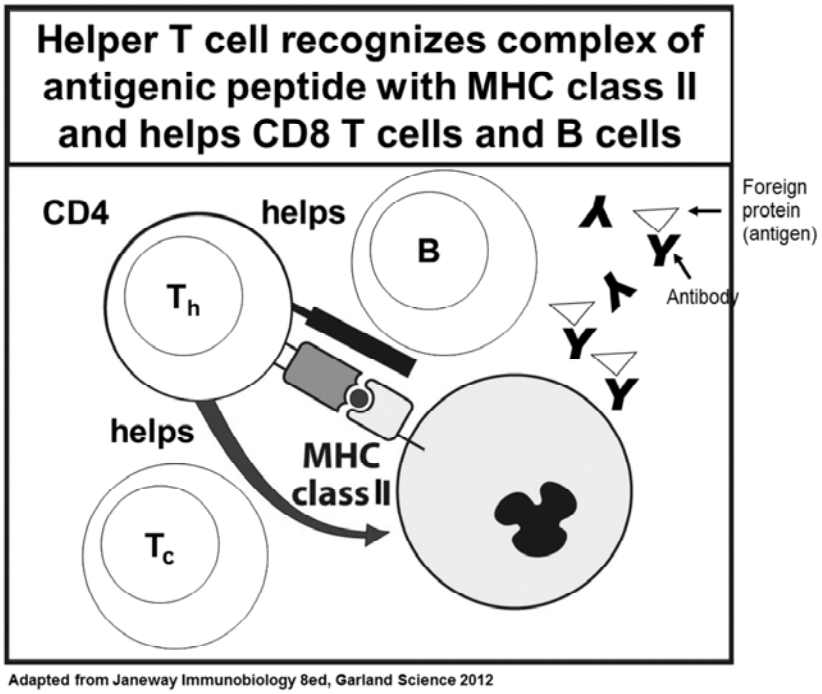

That is contrast with CD4 cells, so we commonly or some think of one fragment of them is helper of T cells because they help to build the immune response. If that is shown that MHC class II with an antigen relevance to the T cell receptor, you can then get secretion of different cytokines which can augment the activity of cytotoxic T cells as well as B cells to make more antibodies,

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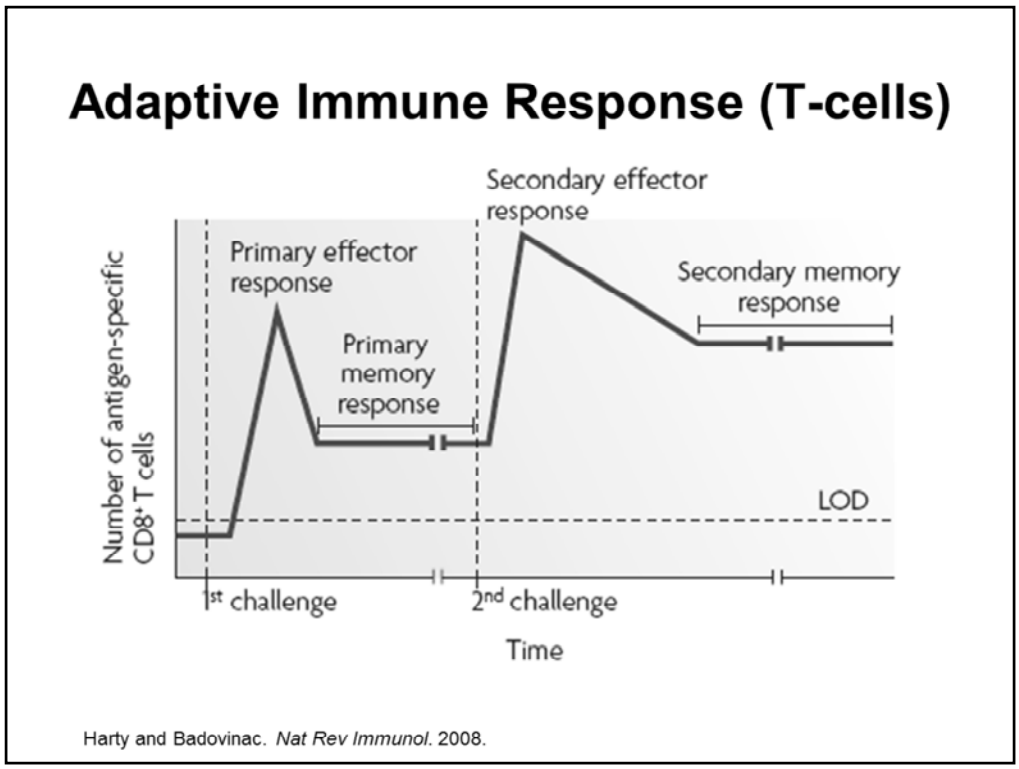

and similarly to the B cell response, the T cell response is also adaptive, so here we see first challenge of an infection, perhaps a tumor, and you get a initial spike in the activity of the T cell population. After clearance of the infection, this will go down but not go away such that you develop a memory response to the tumor. Upon second challenge, you then get another spike and again secondary memory, so this can build over time as a safety mechanism against commonly encountered infections.

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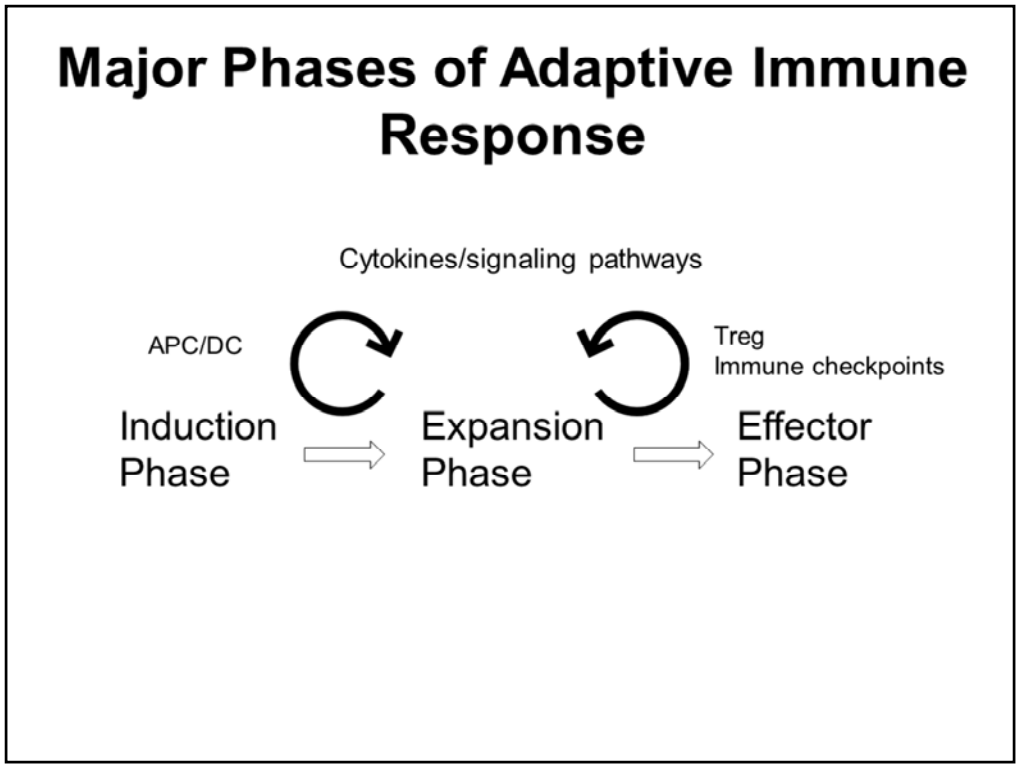

So, the major phases of the adaptive immune response then are the induction phase where molecules are shown to the adaptive immune system by antigen-presenting cells which are commonly dendritic cells as we referred to. They can then undergo an expansion phase. So, after the cells are sort of primed, they start to grow. They are cytokines in different signaling pathways involved, and finally, once they are ready and they are going out to the periphery to try to look for the infection or the tumor per se, to try to fight, you have the effector phase, and each of these can be modulated by different elements,

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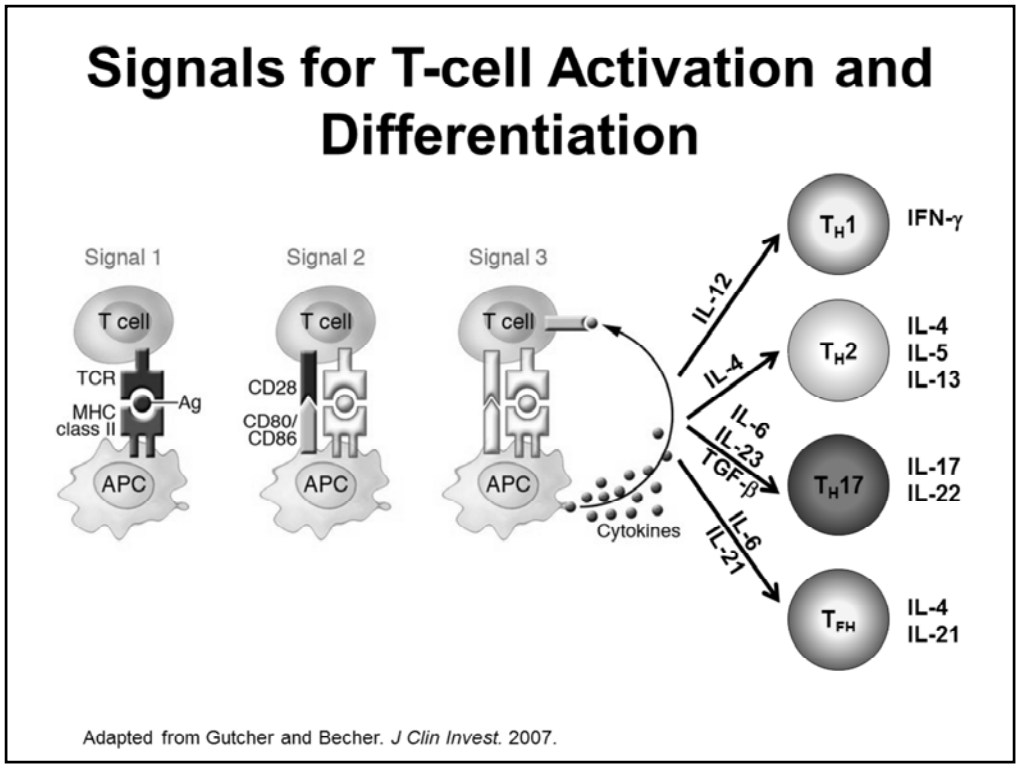

again antigen presentation cytokines and signaling as well as dampening of this effect by T regulatory cells as well as immune checkpoints, and we are going to talk about those at length, and those are probably the reason that you are excited to potentially come this morning.

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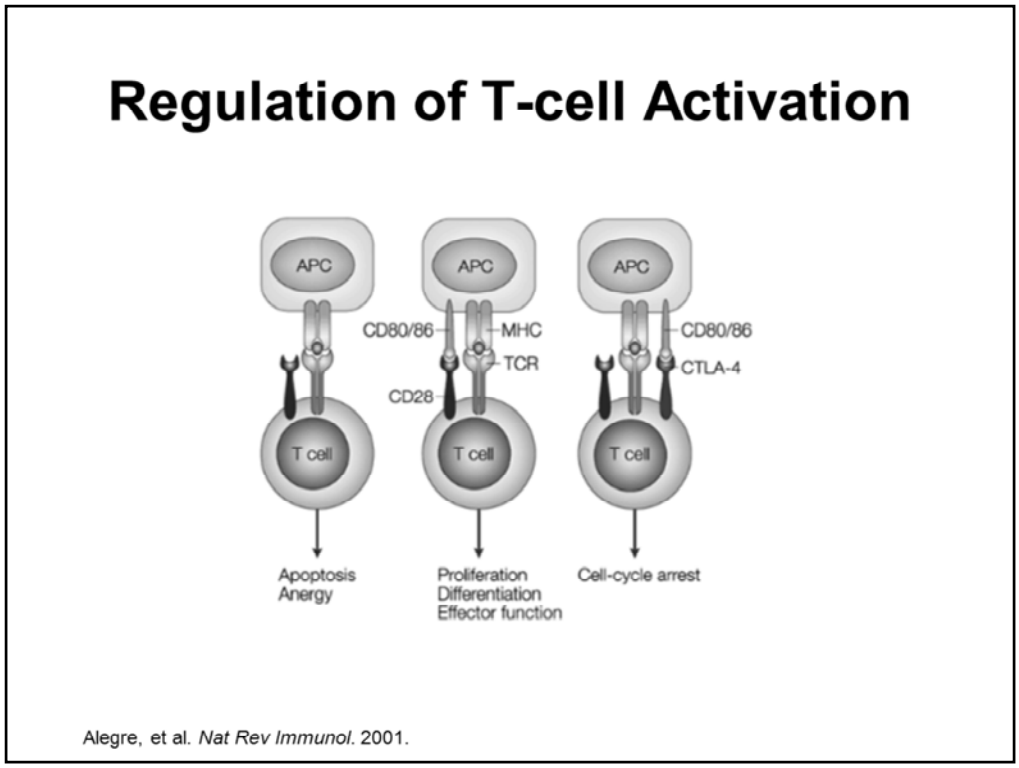

So, how does a T cell get activated? This is a very important process when understanding cancer immunotherapy as well as the development of some drugs over the recent past, and there are three signals that are commonly described, so a T cell has a T cell receptor, it comes in contact with an antigen-presenting cell which is trying to show it something to say, “Hey, is this a problem?” Once it sees the antigen, that is the first signal to start to activate, but that is not enough. If this happens alone, this will actually turn the T cell off and that is to guard against self-reactivity because again, remember that these antigen-presenting cells are floating around your body, just picking up whatever that they come in contact with, so they are going to come in contact with a lot of normal stuff too, and you do not want your immune system attacking the normal things in your body. Therefore, a system developed evolutionary to require the second signal or co-stimulation of the T cell in activation. So, after the T cell has a T cell receptor that sees MHC class II with an antigen, a secondary signal is required here through a molecule called CD28 binding to CD80 or CD86, that then activates the immune cell, and the third signal then is after the T cell is activated it can then secondarily see cytokines released from the antigen-presenting cell and activated cascade of effects through a number of different pathways with secretion of cytokines, etc., to generate different types of responses, and we do not need to go into these at length other than to sort of highlight that in terms of fighting cancer the one we commonly think of as the TH1 response with the secretion of gamma interferons; however, there are others such as the TH2 which is involved with humoral immunity, TH17 with mucosal and epithelial barriers, and T follicular helper which helps to develop further B cell populations.

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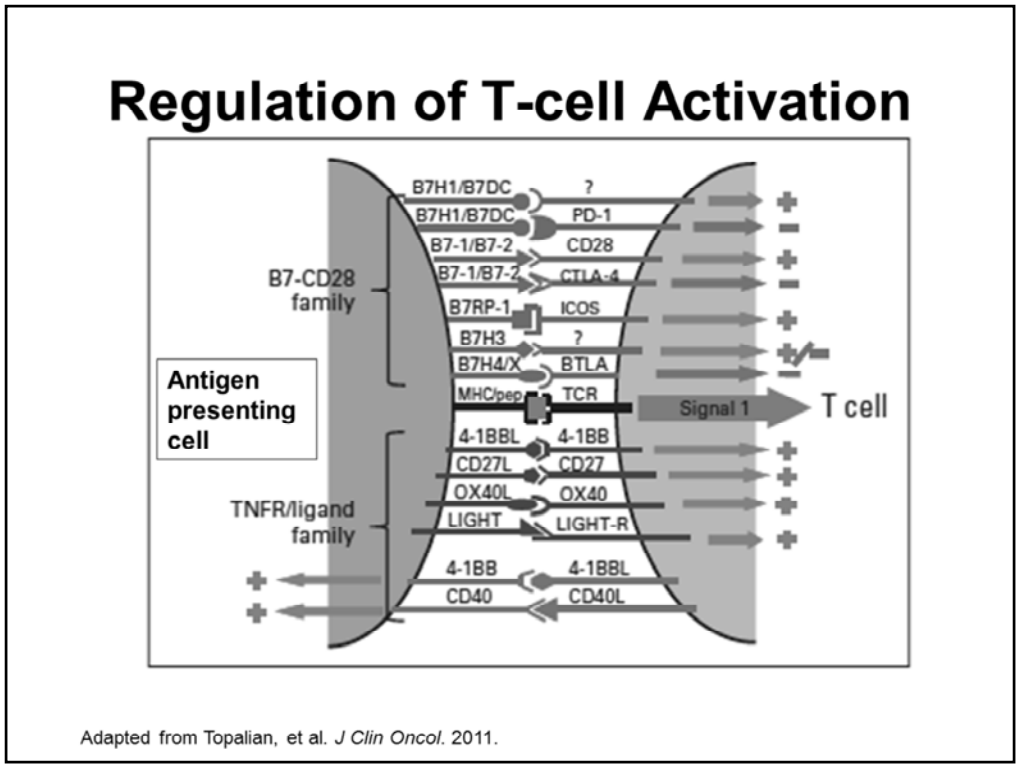

So, in understanding that, then we can start to understand some of the drugs that have been developed so far. Okay, so we talked about the first and second signals within the immune system for activation of a T cell. So, here is your T cell, it has seen an antigen-presenting cell, it has its T cell receptor bound to MHC. It has the second signal here, CD28, that is then engaged by CD80 and CD86, that leads to proliferation differentiating the effector function, but if that was it, we have sort of a runaway immune response every time that it encountered an antigen that generate an overall proliferation of immune cells. The protective mechanism then within the immune response is trafficking of this molecule called CTLA-4 from the Golgi body to the surface of the cell as soon as the cell is turned on. So, you turn on a cell and you immediately start to turn it off, and I tell my patients sort of like circle of life from the Lion King. So, if we do not turn if off then we are going to get out of control. So, what happens is that this secondary stimulate is driving proliferation and growth of these immune cells. CTLA-4 comes to the surface and out competes CD80 and CD86 and turns that site and signal off. Therefore, you stop further proliferation of the immune cell, and as some of you are probably aware, this was identified in the 90s by multiple groups but highlighted by Jim Allison's group at Berkeley, and it was thought what if we can block CTLA-4, could we keep these immune cells on longer? And short story, that is the drug ipilimumab. But that was just a first pass in terms of thinking about regulation of T cells, so that is sort of the simplistic view of how this goes. We are learning actually quite rapidly, meaning on a daily basis, about more of these molecules, these receptors and ligands between immune antigen-presenting cells and T cells that help to modulate this immune response, and I will point out one that is of particular interest likely to most of you in the room, and that would the programmed death-1, PD-1 and PD-L1 interaction, but you can see here, this is just a sampling of different immune ligands and receptors that are potentially modulating. They come in two families, the first two commonly discussed families, and those are the CD28 family and the tumor necrosis factor receptor family. You can see here that some of these ligands and receptors could have positive, meaning to amplify the immune response, and some of them can have inhibitory responses as natural phenomenon. So again, CTLA-4 and PD-1 both dampen the immune response, and that is why giving drugs that block them sort of wake up the immune response, but you can see that there are different ways of doing that, and we are just at the very, very beginning of understanding how we are going to go about doing this, which of these are safe to block, which should we block as single agents, which should we block in combination, and we are going to discuss the development of these drugs and the future of this sort of throughout the rest of the time today.

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How do T cells recognize a cell as cancer?

So, how does a T cell recognize a cell as being a cancer?

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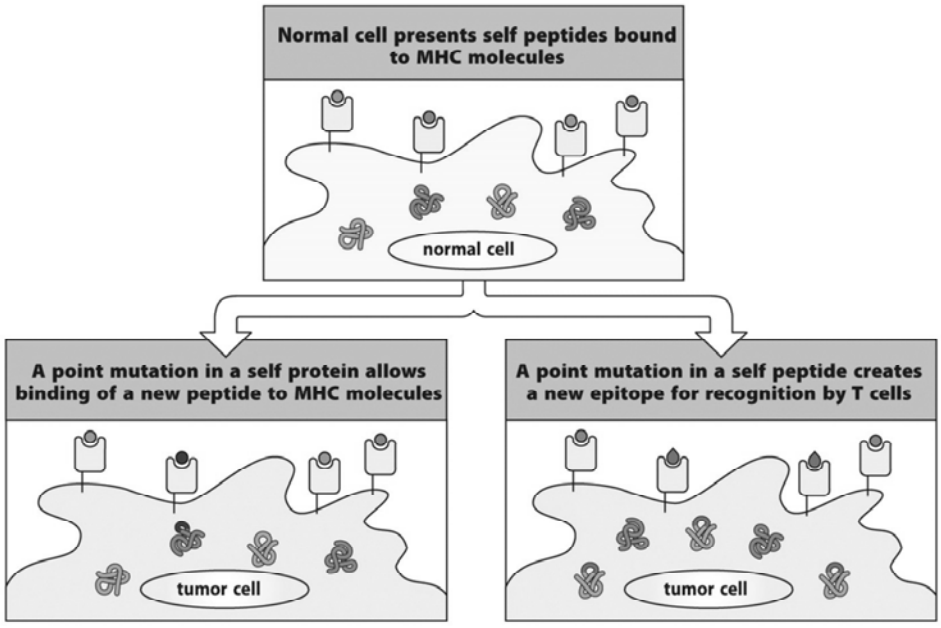

Figure 16.17 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

So, I discussed that in normal cells, you have processing of the proteins within the normal cell to the surface of the cell to show them to the immune system as it is going by. If a mutation happens like in terms of a point mutation creating a new protein, you can see that if this part of the protein perhaps were to be mutated, when it is shown on the surface, it will be slightly different, I do not if this is showing up very well, but this is orange and red and this is red, whereas up here we just have orange and orange. Similarly, down here, a different part of the protein is changed that shows up and just that little change is enough for the immune cell to realize that that is a different protein and it should not be there.

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Potential Tumor Rejection Antigens

Category	Examples
Tumor-specific antigens	Mutated genes (p53, ras), idiotype
Cancer/testis (CT) antigens	NY-ESO-1, MAGE-A3
Differentiation/Tissue-specific	gp100, MART-1, tyrosinase, PSA, PAP,
Overexpressed antigens	WT1, Her-2, MUC-1, survivin, hTERT
Viral	HPV (E6 and E7), EBV (EBNA-3, LMP2)
Oncofetal antigens	CEA, AFP

And there are number of antigens within our bodies that can generate immune responses. These are thrown into the different categories, I am not going to go through that at length, but they are sort of as you can just hypothesize what they might be. If in your tumor you have a mutation in the gene that causes that tumor to grow such as P53 or Ras, that can be immunogenic, but other antigens in your body, say one's that are expressed in the fetal state which are then not expressed during adulthood, they can also induce immune responses such as oncofetal antigens like CEA and cancer testis antigens. So, highlighting cancer testis antigens is also of interest. There are certain areas of our body where immune system, generally speaking, does not go, and so one of them is the testis and the other is in the eye. So, if antigens that are expressed in those areas are then expressed throughout the rest of the body, your immune system can potentially recognize them as abnormal and generate an immune response, and for a long time, for several decades, multiple approaches were taken to try to drive immune responses against these, the expression of these genes, because commonly in cancer with a dysregulation of the genome, you can have aberrant expression of these, which is essentially a non-self protein being expressed in a person.

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Tumor-Host Interactions

- Despite the demonstrated presence of tumor-associated antigens and T-cells capable of recognizing them…
- Most tumors are not controlled by the anti-tumor immune responses…
- Several factors account for this ineffective anti-tumor immunity:
  - Tumor cell-dependent mechanisms
  - Immune cell-dependent mechanisms
  - Microenvironment-dependent mechanisms

So, despite the presence of tumor-associated antigens and T cells capable of recognizing them, obviously tumors are not usually controlled by the antitumor immune response. Otherwise, we would not really be talking about cancer in the first place if this worked every single time. So, there are several factors that account for this ineffective antitumor immunity, and those include tumor cell-dependent mechanisms, immune cell-dependent mechanisms, and microenvironmental-dependent mechanisms. We will discuss some of them here going forward.

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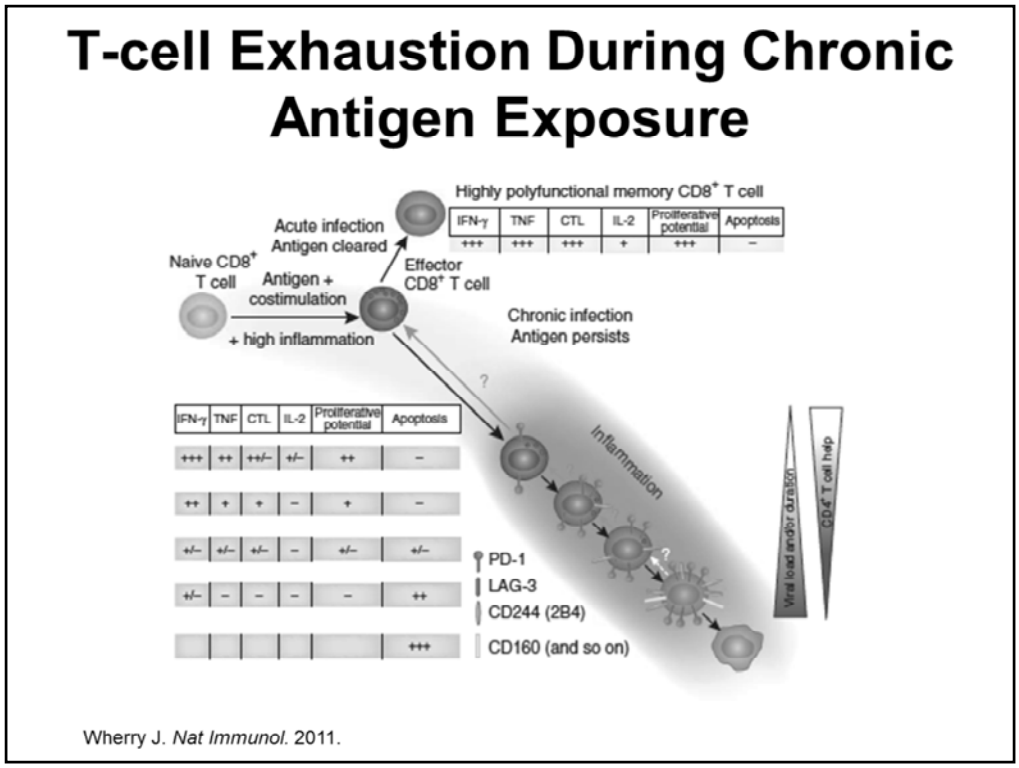

Wherry J. *Nat Immunol.* 2011.

One of the most important in understanding the current development of therapeutics for cancer via immunotherapy is understanding this concept of T cell exhaustion, and this is a term that is under debate within the literature, but I will explain it to you and then I think you will see why we are starting to understanding it may not be the best way to describe it. Here, we have naïve T cell. It has a specificity, but it has not been stimulated yet. It comes in contact with an antigen and it is co-stimulated. It then goes forward and tries to kill off whatever infection that it finds. If it is successful, it can actually go into a resting state or generate a memory response of further CD8 T cells. That is what we hope happens and that is what happens after most infections, but say in the situation of an infection that does not get cleared, and we have chronic exposure to an antigen, that T cell then actually starts to become nonfunctional. So, as inflammation develops and the T cell is not cleared, the natural response for the immune system is actually to start to downregulate so as not to damage your own body. This is what happens commonly in rheumatologic problems. So, as inflammation increases, you start to get expression of some of those receptors that I referred to a little bit earlier. One of the most important is expression of this molecular called programmed death-1, PD-1. Other important molecules are LAG-3, CD244, and CD60. We do not need to go into the details of exactly what these are other than to say they're potential targets for immunotherapy. As the cell expresses more of these and becomes more dysfunctional, eventually, they are driven toward apoptosis or cell death, and you can see that the activity of the cell goes down proportionately so that the active cell which would kill the infection secretes a lot of gamma interferon, has a lot of cytolytic activity, but over time, that goes down. Similarly, the proliferative potential of these cells goes down, and a likelihood of apoptosis goes up. So, chronic exposure within the immune system will actually dampen the ability of immune cells to respond, and we understand that now with the development of the anti-PD-1 antibodies. These are cells that found the tumor but do not appear to be doing their job because they have been down regulated within the tumor microenvironment.

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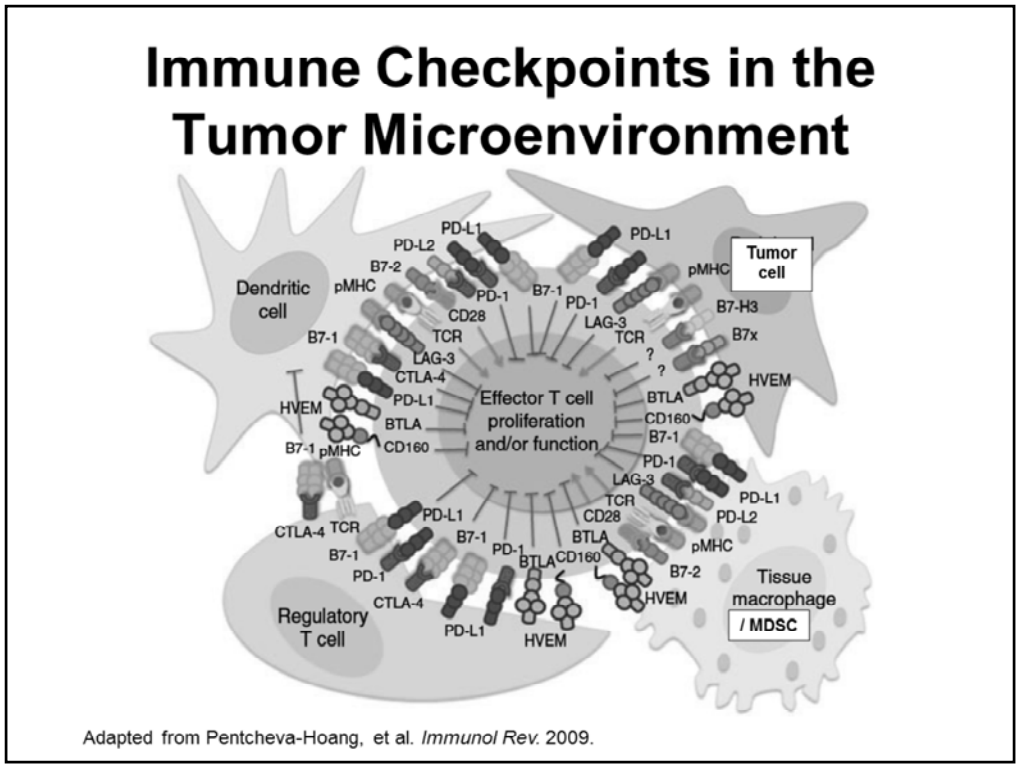

So, to go back again to immune checkpoints, this is again to highlight that we are just at the very beginning of this, so here we have our effector T cell, and it can be modified in many different ways. So, the one we commonly think of here would be through the tumor. You have these different ligands and receptors that are impacting, but you can also see that all the rest of the immune cells can have an impact on the cell as well. The antigen-presenting cell can express a lot of these ligands and receptors as well. Regulatory T cells can dampen the activity of an immune cell. Even the macrophages or part of the innate immunity can impact on these as well. So, it is a very complicated environment, and again, this is why we are just at the very beginning of trying to understand how all of this is going the impact of the clinical management of patients with immunotherapy.

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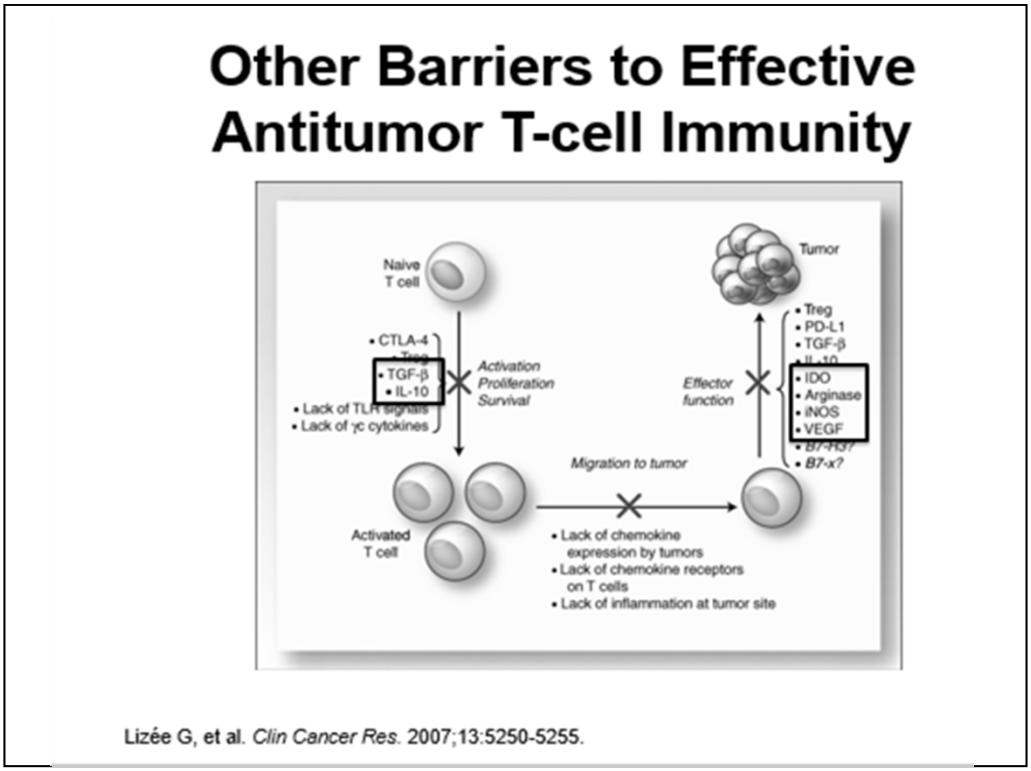

Other barriers to effective anti-tumor T cell immunity include factors affecting the activation, proliferation, or survival of the naïve T cells. The naïve T cells need to be activated after exposure to their cognate ligand, that can be blocked by different cytokines, and important ones to be aware of are TGF beta and IL10. We can have disruption of migration of these activated T cells to potentially their site of activity, and that can be dampened by reduction in the expression of chemokines which are chemicals which help to track certain immune populations into their appropriate site whether it be a tumor, an infection, or just a ceratin part of the body, and finally, once immune cells get to the tumor, they also could be dampened by factors secreted within the tumor microenvironment. So, it turns out that T lymphocytes are highly dependent on a certain concentration and homeostasis of tryptophan within the body, so modulation of tryptophan concentration through the enzyme indoleamine 2, 3-dioxygenase is a very interesting aspect of cancer immunotherapy. It is also a real good joke to use with your significant others during the Thanksgiving, especially if you are sort of an immunotherapy dork like me. I am talking about should we just eat more turkey and rule out impact on cancer, but other factors eventually, you are all probably aware of VEGF or vascular endothelial growth factors, nitric oxide can impact on the trafficking as well as activity of immune cells. In addition, arginine metabolism is quite important.

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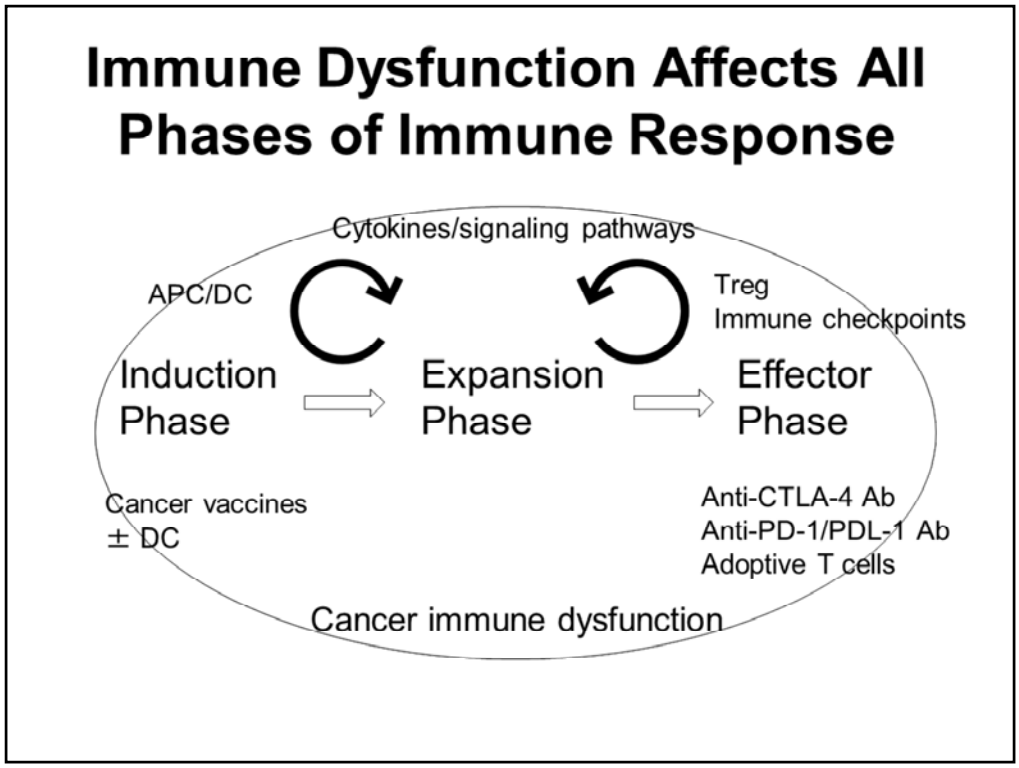

So, if we again think about the phases of the immune response, you can have dysfunction that impacts on potentially all of these. So, in the induction phase, we talked about antigen-presenting cells, expansion with cytokines, effector phase with T RAGs, and immune checkpoints. There are different ways to go about trying to address this as well. Could we try to prime the induction phase better, and so how could we do that? Well, we could think about a cancer vaccine which would help to tell the immune system what to go after. We could think about different immune checkpoint inhibitors to try to take the brakes off so to say of some of those blocking immune checkpoints, and some of these have been developed such as I said anti-CTLA-4, anti-PD-1, anti-PD-L1 or could we even transfer in cells that already know what to look for, and that is known as adaptive cellular transfer, and we will talk about that near the end of the talk.

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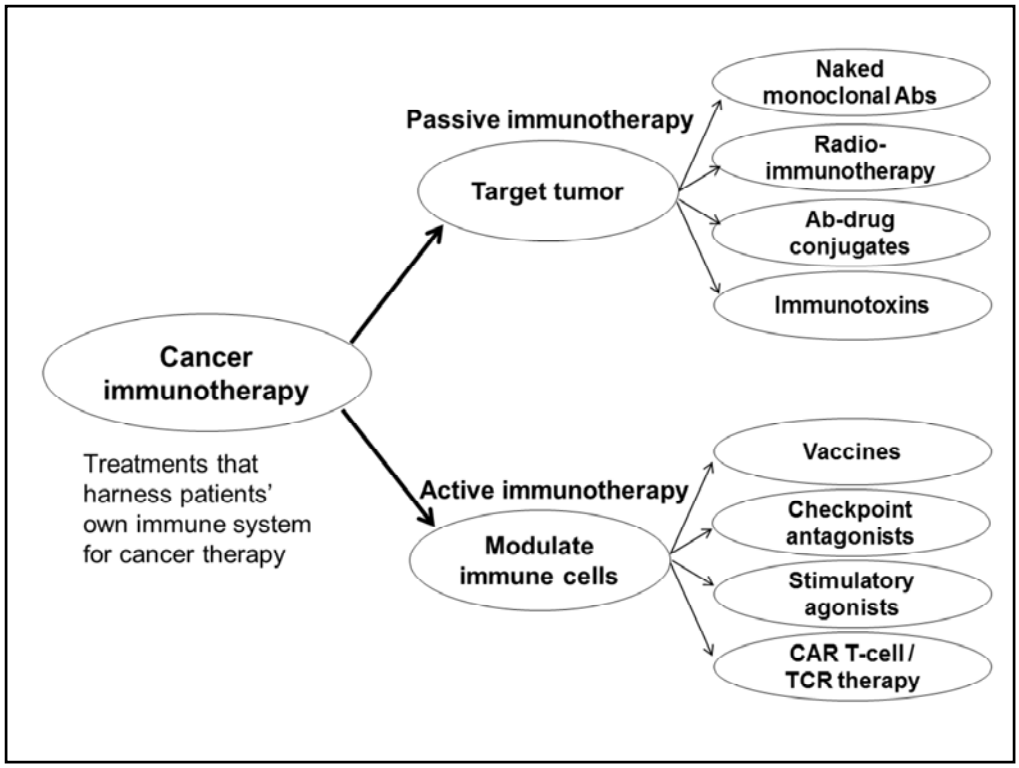

So, let's us go back now to where we started in thinking about passive versus active immunotherapies and talk about more specific examples, so passive immunotherapy could include naked monolocular antibodies.

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Tumor tissue origin	Type of antigen	Antigen	Tumor type
Lymphoma/ leukemia	Differentiation antigen	CD5 Idiotype CD52 (CAMPATH1)	T-cell lymphoma B-cell lymphoma T- and B-cell lymphoma/ leukemia
	B-cell signaling receptor	CD20	Non-Hodgkin's B-cell lymphoma
Solid tumors	Cell-surface antigens Glycoprotein Carbohydrate	CEA, mucin-1 Lewis^y CA-125	Epithelial tumors (breast, colon, lung) Epithelial tumors Ovarian carcinoma
	Growth factor receptors	Epidermal growth factor receptor HER-2/neu IL-2 receptor Vascular endothelial growth factor (VEGF)	Lung, breast, head, and neck tumors Breast, ovarian tumors T- and B-cell tumors Colon cancer Lung, prostate, breast
	Stromal extracellular antigen	FAP-α Tenascin Metalloproteinases	Epithelial tumors Glioblastoma multiforme Epithelial tumors

Figure 16.19 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

These can be against the host of different targets, and I will not go through this at length other than to sort of highlight that many of them have been developed against growth factors to date, so you are all aware of epidermal growth factor receptor antibody for various different tumors such as cetuximab and panitumumab, HER2-directed such as trastuzumab, VEGF-directed such as bevacizumab, and these can be developed sort of across the board.

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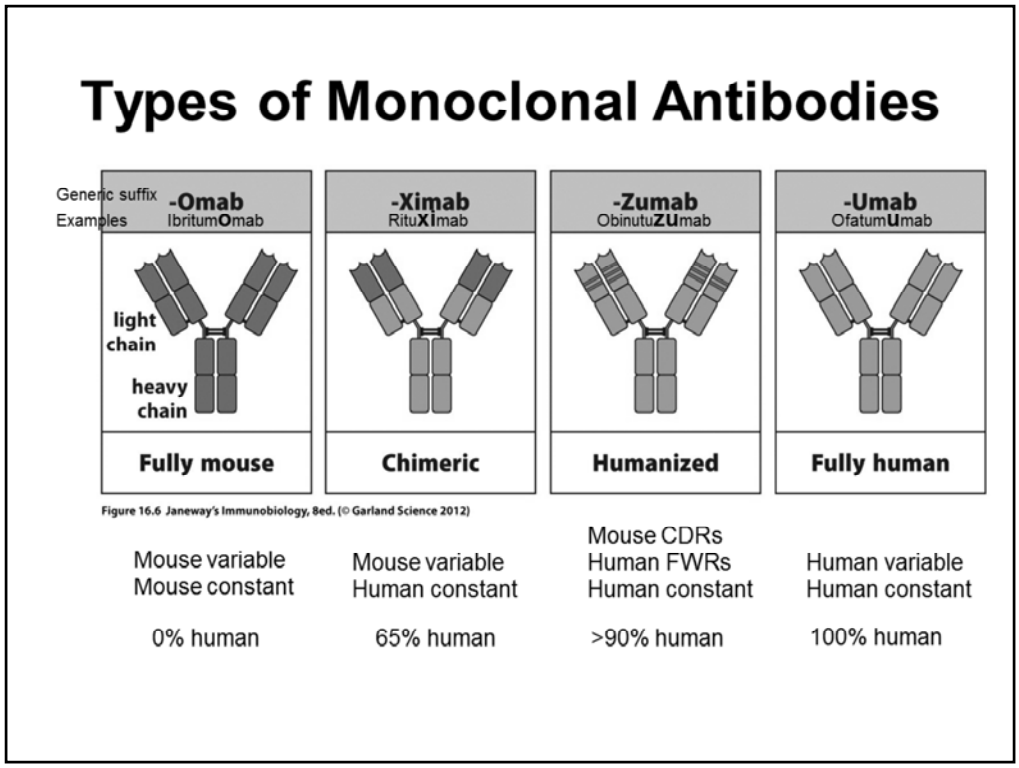

So commonly these drugs are developed in preclinical models and then transitioned into humans, and so the nomenclature is interesting and important to understand. So, when the antibody is first developed within a murine or mouse system, it is fully mouse, and you can see that it ends in an –omab, so ibritumomab. It is not a human antibody, and that has consequences because that is a foreign antigen. So, the antibody itself they are using therapeutically can generate an immune response, and that can limit the effectiveness of the drug. If you transition then a chimeric antibody, ends in –ximab, that has components of the mouse as well as the human, approximately 65% human, keep going. You have a molecule that is humanized, meaning it is now greater than 90% human, and only portions of it is still retained in the mouse DNA sequence, and that ends in –zumab, and finally fully human antibodies ending in –umab are 100% human. These are the ones that are, generally speaking, the most efficacious because they are least likely to generate an immune response specifically against the drug.

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Mechanism of Action of mAbs

- Antibody-dependent cell-mediated cytotoxicity (ADCC)
- Antibody-dependent cellular phagocytosis (ADCP)
- Complement-dependent cytotoxicity (CDC)
- Direct apoptosis
- Agonistic – induce signaling
- Antagonistic – block receptor-ligand interaction

These drugs could have different mechanisms of activity, so you probably remember ADCC from medical school or other CME programs when an antibody binds cells can come in and stick to the back of the antibody, the Fc portion to generate these different approaches to killing infections or tumors, so antibody-dependent cell mediated cytotoxicity, cellular phagocytosis, generation of complement, directly inducing apoptosis agonistic, or antagonistic interactions,

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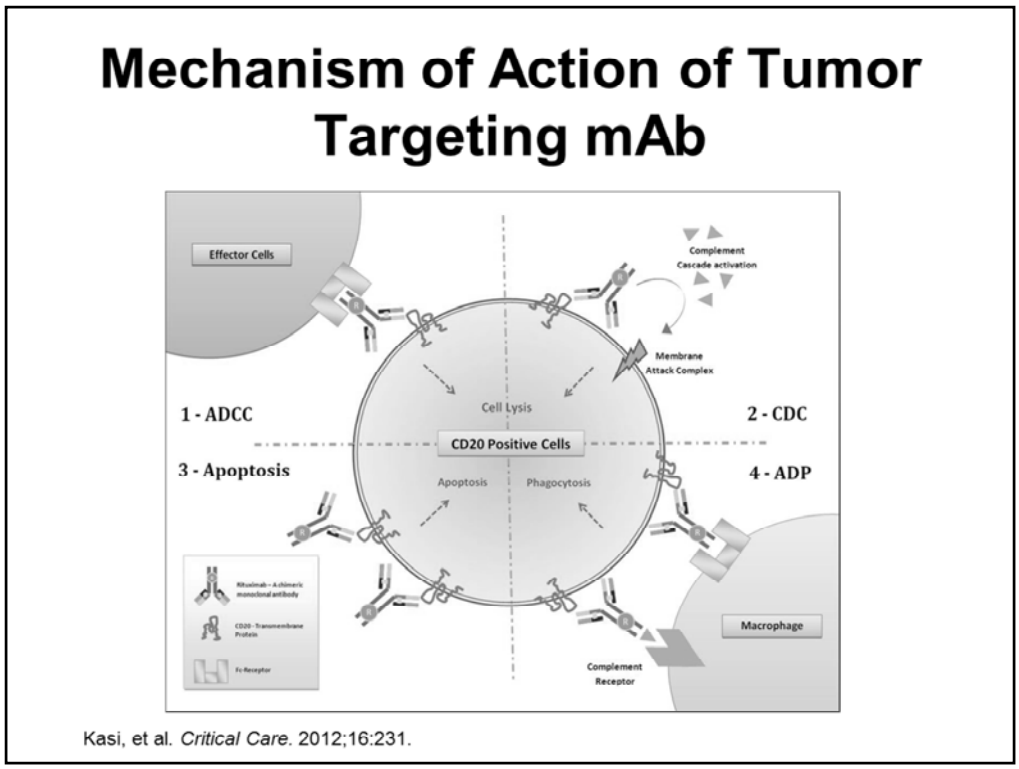

and here is a schematic of that, so if we have an effector cell, the antibody comes and binds, it can then recruit other cells to come in to lyse that cell. Alternatively, you can have the antibody bind and generate a complement reaction, which then potentially could kill the cell. You could have phagocytosis or just generally by blocking the receptor or whatever the antigen is, you can generate an apoptosis response within the tumor.

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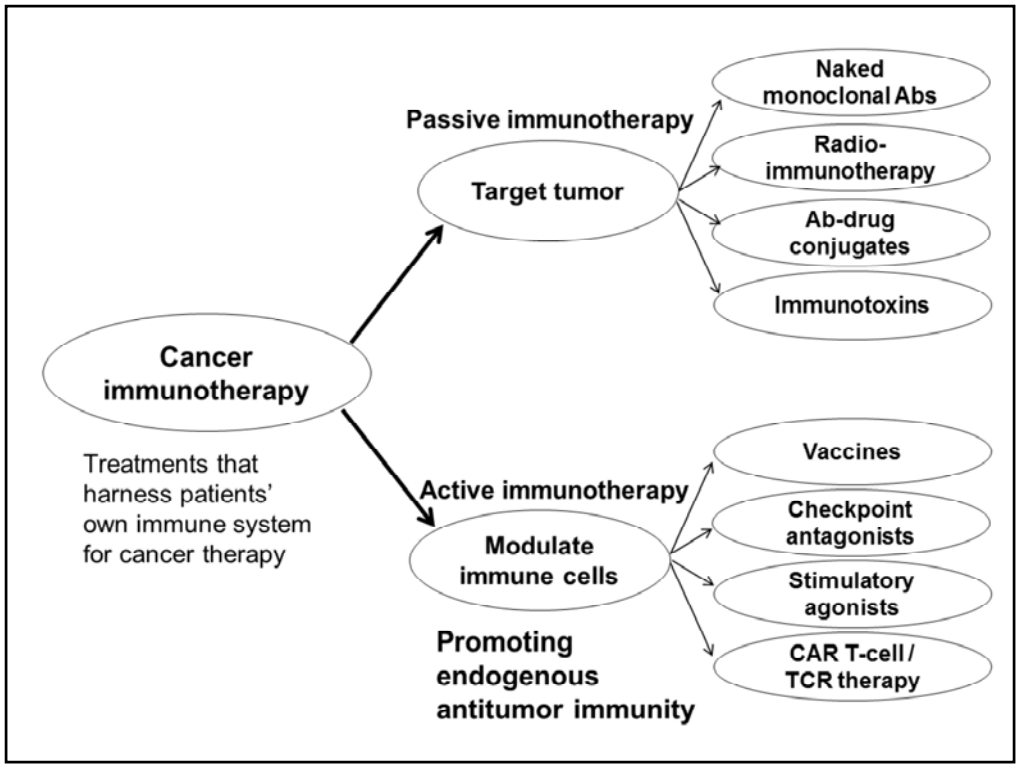

So, now going back and thinking about cancer immunotherapy on the active immunotherapy side, again the idea here is that we are going to modulate the immune response to get a bigger immune response against the tumor, and what we are going to talk about here are checkpoint antagonists and stimulatory agonists. So, I alluded to this earlier, but there are these different receptors and ligand interactions within the tumor microenvironment and within different immune populations, and they can have different effects.

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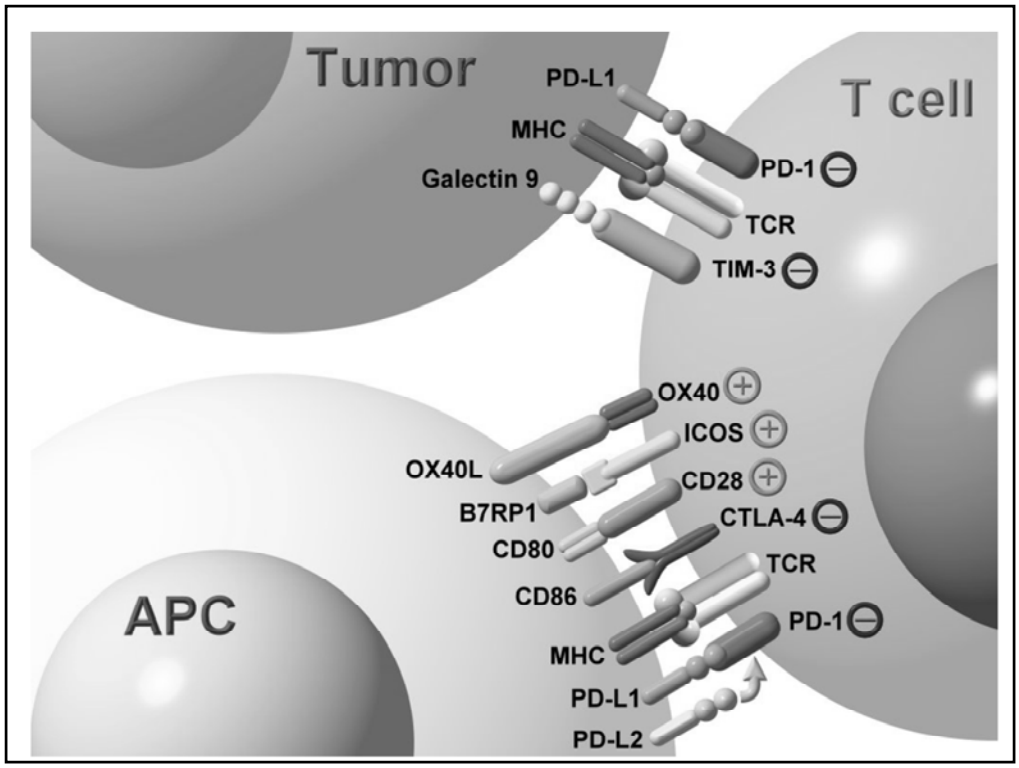

So, here we see the interaction I talked about near the beginning between CTLA-4 out competing CD27 for CD86. That is an inhibitory interaction that turns the immune cell off after it is initially activated. So, blocking that then could release that inhibitory activity. Similarly, the PD-L1, PD-1 interaction is an inhibitory interaction and that then by blocking it can relieve the suppression of those cells and potentially release antitumor immunity. There are a number others, however, that are in clinical development and I will highlight them because they may come into clinical relevance in the next few years. OX40 is an agonistic meaning when you bind this you drive the immune response, so you if gave agonistic antibody or antibody that would bind and push the immune response, that way you could actually overdrive the immune response. Similarly, ICOS is such a molecule, CD28 we have talked about a few times, so obviously if you push it through CD28, you will get more response. There are other inhibitory molecules, so here is one called TIM-3. There is another very important one called LAG-3 that is in development that you may see relatively soon as well.

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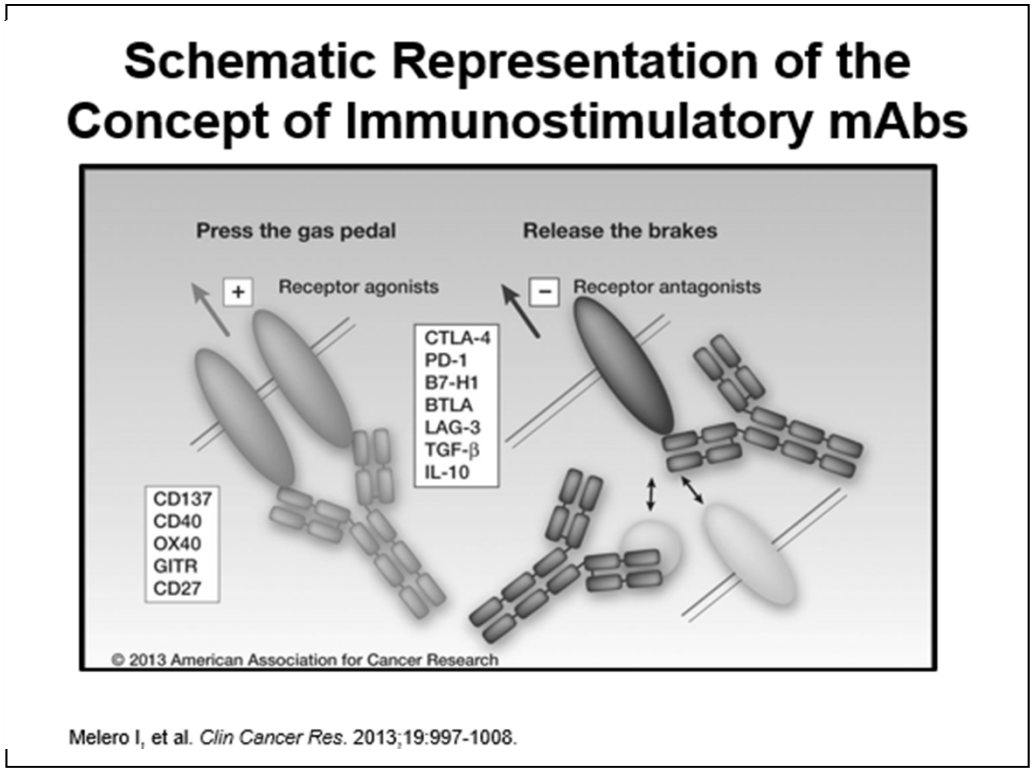

So, here is a schematic representation of how this works. Again, for an antagonistic receptor, if it is bound, it will then downstream shut things off, and here are examples of that CTLA-4, PD-1 like I said, whereas an agnostic receptor will drive signal, and examples of that here, CD137, CD40, CD27, OX40, etc.

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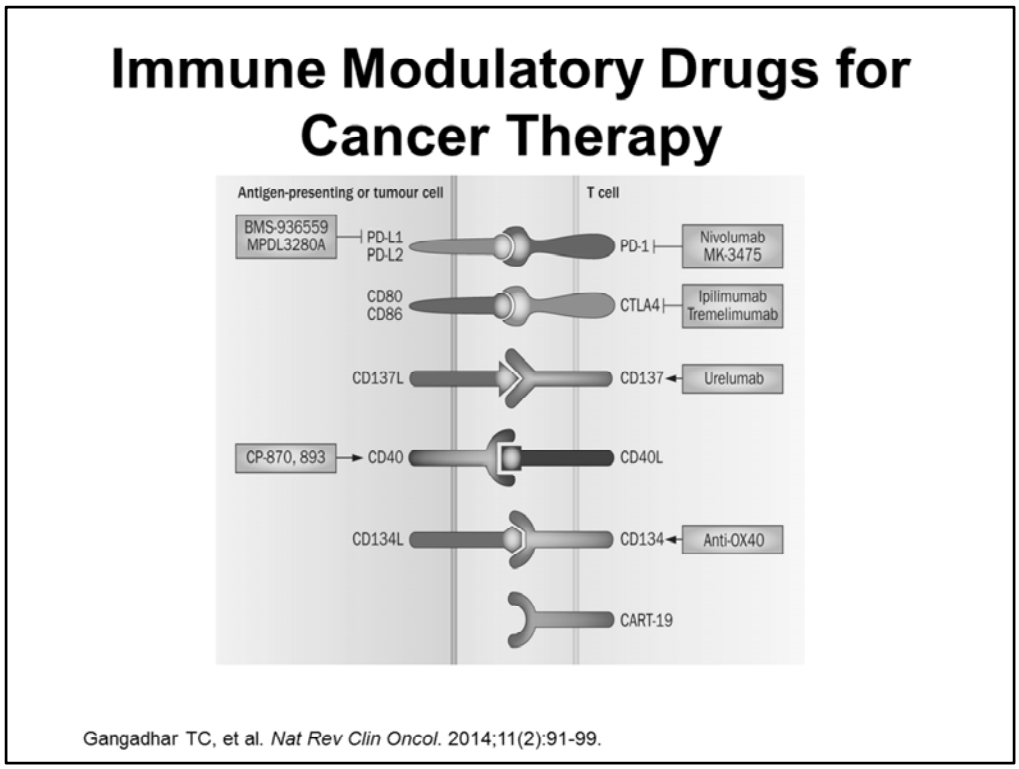

So, immune modulatory drugs for cancer therapy are now in development or actually in clinical practice, and that is predominantly why you are probably here today. So, we know for anti-CTLA-4, the drug is ipilimumab which is approved for melanoma. There is a sort of sister compound called tremelimumab which actually is not approved but likely is of similar efficacy. On the PD-1 axis, we have anti-PD-1 antibodies such as nivolumab and MK-3475 or pembrolizumab. On PD-L1, PD-L2 side of things, we have a BMS compound 936559 which is no longer being developed but more importantly MPDL 3280A which many of you have probably heard of for bladder cancer, but other molecules are also being drugs, so CD-137 there is a drug called urelumab. They are anti-CD40 antibodies. There are anti-CD134, anti-OX40 antibodies in development as well.

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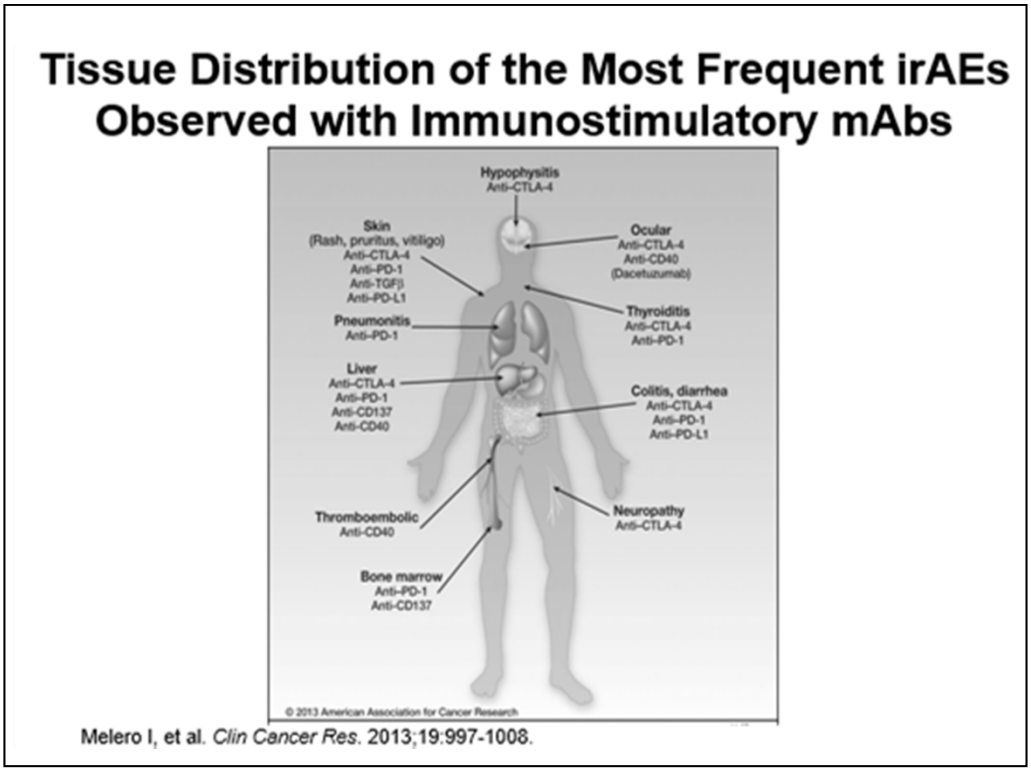

Understanding the specifics of these molecules is important when we think about actually treating patients. So, you would think, well this is all the immune response, therefore, you are going to get the same thing with every single one of these drugs, but the issue is that you get different modifications of the immune response, more and less response, and what we have already observed is that giving different antibodies gives a different intensity of the potential for side effects. So with anti-CTLA-4 antibodies, the primary toxicity that we have been concerned about to date has been colitis and diarrhea, that can be seen with other immunomodulatory molecules, but it is less frequent. With molecules like anti-CD40, we have seen more ocular sort of consequences. All of these different inflammatory modulating molecules can cause skin effects so on and so forth such that watching each patient and understanding the kinetics of how side effects develop with each one of these agents is particularly important as they are quite different from standard chemotherapies.

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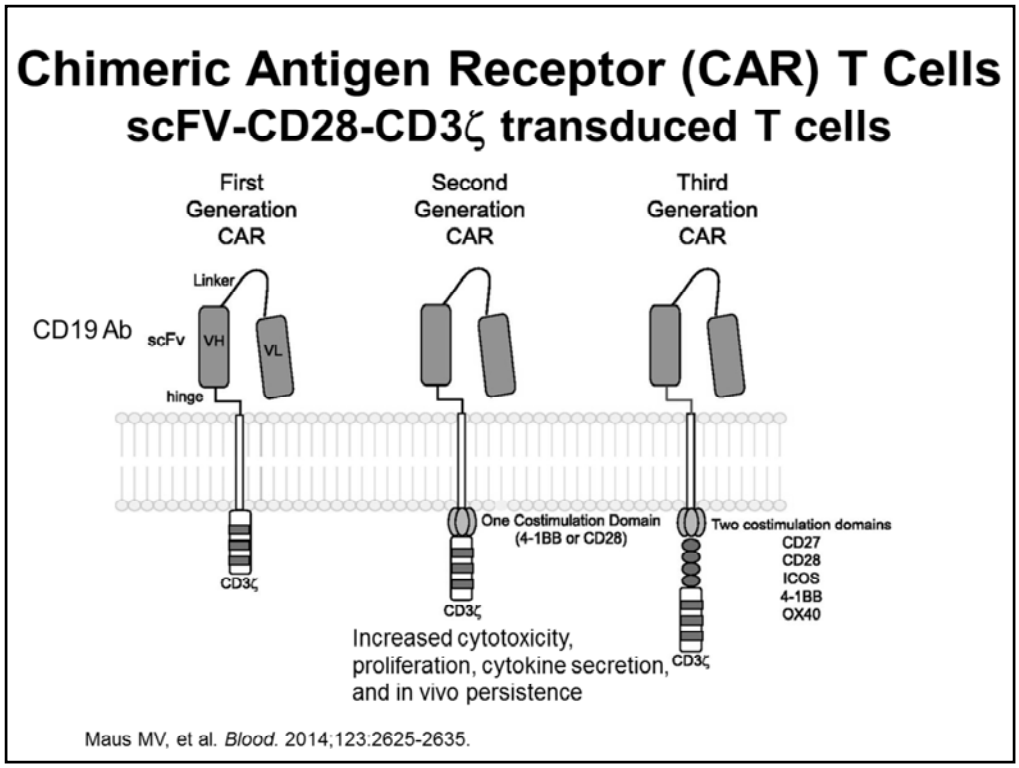

So, in the end here, we are going to talk a little bit about CAR T cells or TCR therapy. This is something you should definitely be aware of, which may be coming into clinical practice relatively soon. What is a CAR T-cell? It is a chimeric antigen receptor T cell, and essentially, this is sort of a fusion protein where we hope are the best of all pieces of an immune cell and engineer it so that it knows to go to fight the tumor. So, when you look at it into schematic, it may look confusing, but you realize you have seen many of these parts before. These molecules are made up of a side chain of variable fragments. So, again, these are parts of the antibody or the T-cell receptor which can then bind to an antigen here. They are then linked through the CD-3 data chain which is here. This is the linker down through the membrane and then within the molecule are introduced activation motifs to try to drive the cell and do its thing without any stimulation. So, we just talked a little bit about CD28 which is a costimulatory molecule, but there are several others, and you may also notice that we talked just a little bit previously OX40, 4-1BB, ICOS, CD27, etc. So, you can engineer in more or less stimulation for these cells such that when you give them to patients they may immediately go and do their thing. They do not need to be stimulated by any immune response that already exists. Now, the problem with that is you put them in and they go do their thing, and so if do their thing a little more than you want, you have to be very careful about the generation of inflammatory side effects, but this is an area of active investigation that has looked quite promising in hematologic malignancies. At ASH recently, they presented followup data in ALL showing that patients who had progressed through nearly all lines of therapy, something in the order of 80% or 85% of them are essentially what appears to be cured of their ALL. Now, there were some various serious toxicities associated with this. So, this is just the beginning of this, and we are learning how to harness this potential moving forward.

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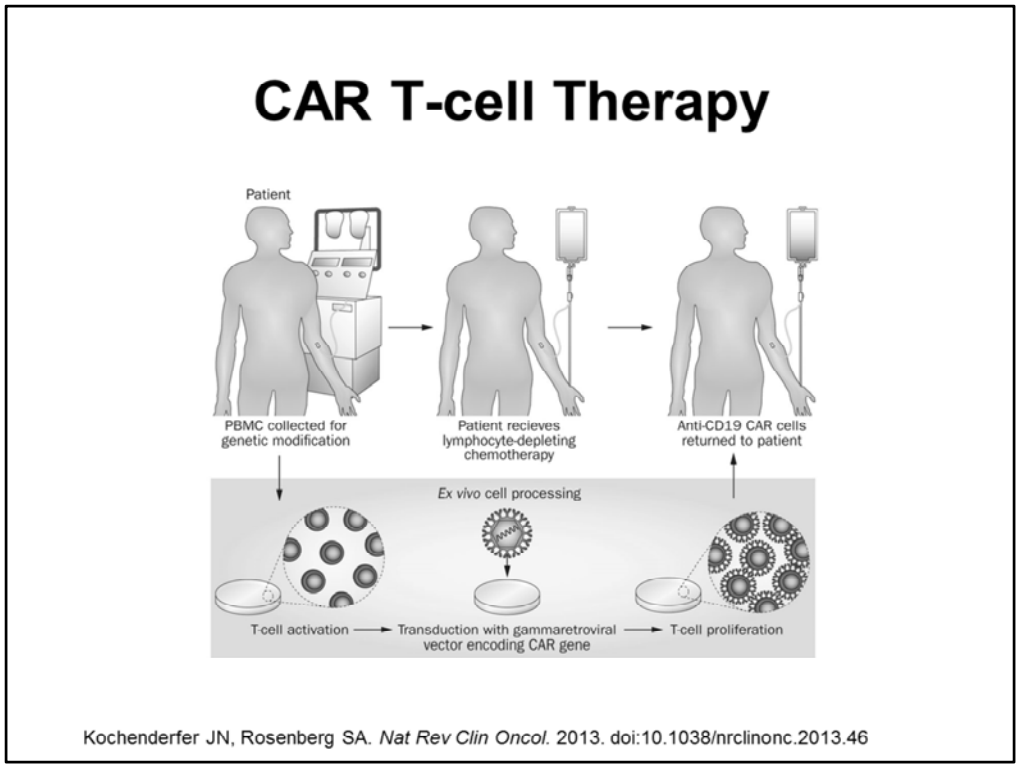

How this CAR T-cell work practically speaking? Well you take the patient, you collect some of their peripheral blood, mononuclear cells, you then can modify them by introducing these genes through a number of different mechanisms, although the most common is by introducing a gamma retrovirus which then brings that gene into the cell, you can then give chemotherapy to the patient to get rid off their immune cells and then give them back these anti whatever you are interested, in this case CD19 cells, which can then immediately go and look for the tumor and potentially kill the tumor.

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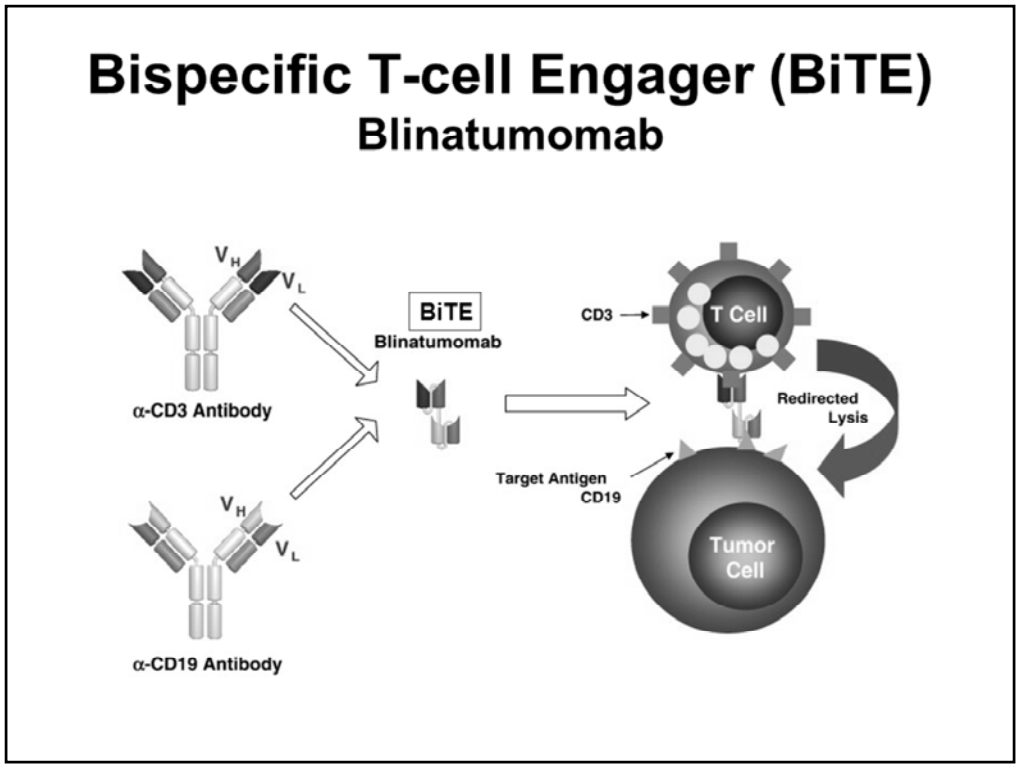

A somewhat similar sort of variation on this is a technology known as bispecific T-cell engagement technology or BiTE technology, and this drug was also recently approved by FDA for the treatment of the same population of patients. What are these drugs? Well essentially, remember we talked about these antibodies and what we want them to do is bind to things potentially to send signals. Well what if we could just take off the parts that bind to things and then hook them up with something that binds to bring two things together? That is essentially what we are doing here. So, if you take an anti-CD-3 which is for T cells and then you link the part that binds the T cell to an anti-CD19 antibody, the part that binds to the tumor cell, you just put them together. Basically, what you will do is you will get one side hooking on to the tumor cell, the other side hooking on to the immune cell that you want. That then brings the immune response basically directly to the tumor, and this treatment has also been highly efficacious in very advanced patients. It is now available actually in the standard practice.

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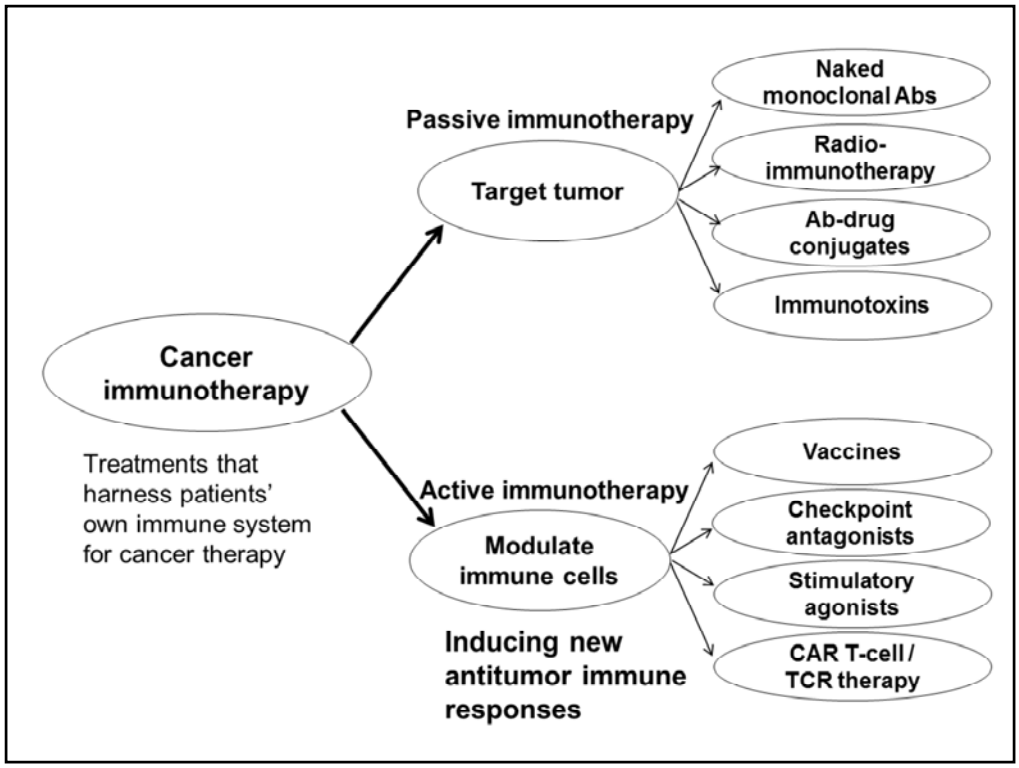

So, finally I am going to just touch briefly on cancer vaccines. The idea here would be to direct the immune response by showing it what you want the immune response to go after. You have been probably aware of cancer vaccines for a long time. They are quite different than infectious disease vaccines however, so whereas with infectious diseases, we vaccinate people before they get sick so their immune system is aware of a potential infection when they see it, but by definition, for cancer vaccines we are vaccinating somebody who already has been exposed and has already essentially had a failure of their immune response in going after the tumor.

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Infectious Disease vs Cancer Vaccines

Infectious disease vaccines

- Used for prevention of infectious diseases – flu vaccine, polio vaccine, small pox vaccine.

Cancer vaccines

- Used for treatment rather than prevention of cancer – only one FDA-approved therapeutic cancer vaccine – Provenge for prostate cancer.
- Two infectious disease vaccines can prevent cancer – eg: HPV, hepatitis B

So, infectious disease vaccines are used for prevention, and here are some examples. Cancer vaccines are used for treatment, and to date, there is only one approved which is the sipuleucel-T that we are going to hear more about here a little bit. There are some vaccines against infectious agents which can also be protective against cancer. Examples are HPV and hepatitis B and you are aware of those and I will not belabor that.

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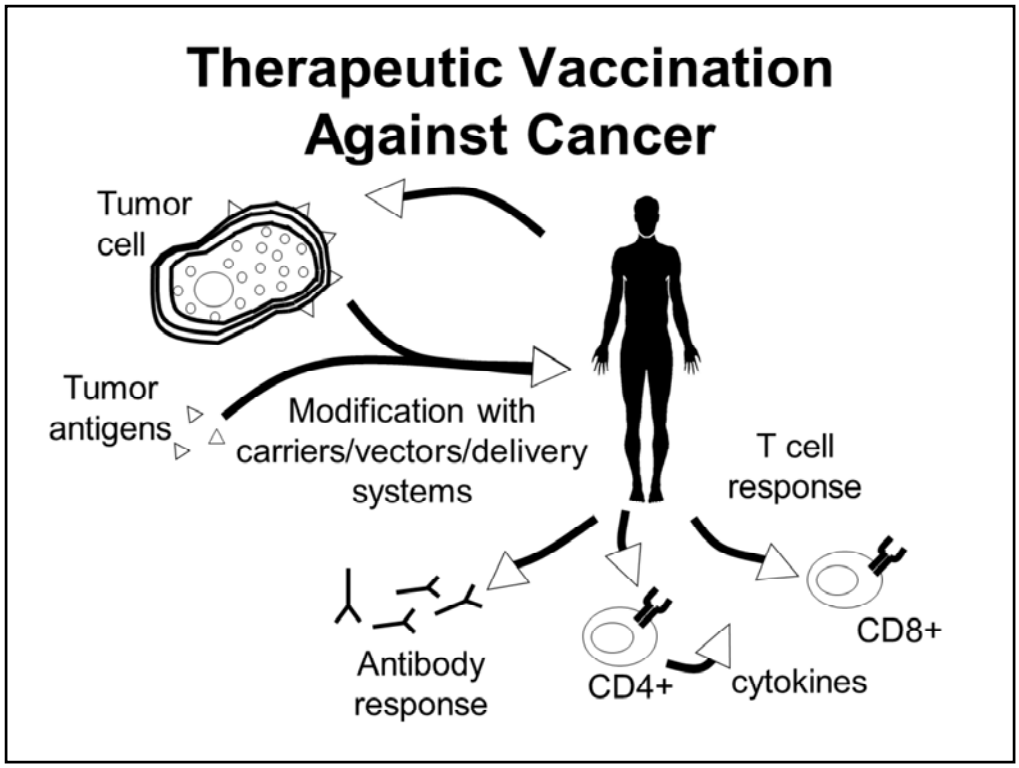

The idea behind therapeutic cancer vaccination is that we can introduce elements from a tumor cell, give them back to a patient which could preemptively generate all the immune characteristics I referred to before, the generation of antibodies to bind to the tumor, the development of CD4 positive cells that develop cytokines to amplify the immune response as well as hopefully CD8 cells which can eventually have effector function.

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Components of a Cancer Vaccine

- **Antigen** Specific to the tumor
  - short peptide
  - long peptide
  - protein
  - gene (plasmid DNA, viral vector, bacterial vector)
  - primary tumor cells or tumor cell lines
  - Ag-loaded dendritic cells
- **Carrier** Needed for peptide or protein formulations
  - protect from immediate degradation (IFA)
  - provide slow release of Ag (IFA, liposomes)
  - facilitate transport to lymph nodes (liposomes)
  - facilitate uptake by APCs (KLH, DEC205, chemokines)
- **Adjuvant** Immunostimulatory
  - provide danger signals (TLR ligands – CpG, MPL)
  - recruit and/or maturation of DCs (GM-CSF, CD40L)
  - T-cell growth factor (IL-2, IL-15)

So, the components of a vaccine, we are likely all aware of this, has to be the antigen what you wanted to go after. You can have various different possibilities here. It could be certain short or long peptides, proteins, certain genes. What about the carrier? Meaning how you are going to get it, how you are going to keep it there so that the immune system can see it? And there are various different peptides predominantly those that are highly immunogenic which are going to track in immune cells to show this antigen to them and you need an adjuvant again to help to generate an immune response that is going to track in different immune populations.

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Randomized Cancer Vaccine Trials Showing Clinical Efficacy

	Sipuleucel-T	BiovaxID	gp100	PROSTVAC-VF
Phase	III	III	III	II
Type	Cellular	Protein	Peptide	Viral
Formulation	APC + PAP-GM-CSF	Id-KLH + GM-CSF	gp100 + IFA + IL-2	Poxvirus-PSA+B7.1/ICAM-1/LFA-3
Disease	Prostate cancer	Follicular lymphoma	Melanoma	Prostate cancer
Improvement in OS	4.1 mo	N/A	6.7 mo (NS)	8.5 mo
Improvement in DFS/PFS	None	13.6 mo	0.6 mo	None
Response rates	2.6% vs 1.3%	N/A	16% vs 6%	1% vs 0%
Reference	*Kantoff et al, NEJM, 2010*	*Schuster et al, J Clin Oncol, 2011*	*Schwartzentruber et al, NEJM, 2011*	*Kantoff et al, J Clin Oncol, 2010*

There have been some randomized clinical trials of cancer vaccines which unfortunately have not been particularly successful yet to date. The one that has been, the sipuleucel-T, again, this is a dendritic cell vaccine where patient's blood cells are taken out and then pulsed with antigens from the tumor and then given back to the patient to help to generate an immune response. There have been different approaches with different antigens, and this is again of current investigation because now we have understood how we can take the brakes off the immune system. We have vaccinated people and shown that we can generate immune responses, but those generated immune responses do not work in order to treat the patient, but now with the development of an immune checkpoint blockade where we can actually in the tumor microenvironment allow those cells to go do their thing. There is a renewed interest in vaccines as to say let's guide the immune system toward what we want and then take the blocking effects of the tumor microenvironment away and allow the immune cells then to go and kill the tumor. So, that is the end of the primer here.

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