DMITRY I. GABRILOVICH, MD, PhD: Hi. My name is Dmitry Gabrilovich. I'm from H. Lee Moffitt Cancer Center in Tampa, Florida. And I will be talking to you about Basic Concepts in Immunology as it applies to lung cancer.

First, we need to discuss briefly the main paradigms in cancer biology and tumor immunology. We all know that tumors are of monoclonal origin, but we also appreciate that the fact that this is heterogeneous cells, and therefore they share the phenotype of cells from which they arose. So that depends on -- If it's epithelial cells, or it's origin from other tissues, we would always trace them.

It's very well known in pathology, but also it's very important for immunology as well, because tumor cells typically express some antigens, and the tumor-specific responses can be induced in cancer patients. Why? Because these antigens arise from the molecules which were mutated or truncated or otherwise overexpressed, which make tumor cells different, not in a major way, but somewhat different from normal cells. And therefore, the immune system can be educated to trace the cells and eliminate them. However, as we all appreciate, the immune system is ineffective in controlling tumors, and therefore something needs to be done to make the immune system able to control tumor progression or, better, reject tumor.

There are two major groups of antigens. One we call tumor-associated antigen, TAA, and that's what probably most of the presentations of mine and other people who will present the clinical results will be focused on. This is a so-called normal antigen which presents in many of the normal cells, but because of the different reasons -- deletion, mutations, functional overexpression -- they reproduce in large amounts in tumor cells. Therefore, they become available for targeting by immune system.

Tumor-specific antigens is the ideal antigen. This antigen presents only in the tumor cells and not in any normal cells. So it's easy to reason that these antigens will be the best target. Unfortunately, there's few examples of the tumor-specific antigens, and none of them really applicable to lung cancer. The best examples of tumor-specific antigens are viral antigens, the one which are caused by viruses. Unfortunately, it's not always the case in many type -- types of cancer, and very rarely in lung cancer.

That will give me the link to the next topic, which are really critically important, especially for the clinical oncology. And it's what will be the ideal tumor antigen or tumor-associated antigen, as we already agree that TAA, tumor-associated antigens, are really the ones which express in lung cancer. So you want to see antigen expressed widely on tumor cells, and at a much higher level than in normal cells, obviously, because you need to have some threshold above which tumor -- the immune system can recognize tumor.

So these tumor-associated antigens should not be relatively rare, and should be expressed in a significant proportion of cancer patients. We all know that if there's a very tiny amount of patients who benefit from this treatment, the treatment will not be very powerful. Although in the future, we all know that we try to select a responder population based on some criterias, and expression of certain TAA will be one of them.

If TAA is part of the cell protein, a potent immune response against it should not lead to severe autoimmune abnormalities and -- as sometimes is the case. Survival of tumor cells is dependent on the presence of molecules containing the chosen TAA. So what it means, it means that the tumor cells targeted by the immune system to -- cannot simply downregulate this TAA, and thus escape immune recognition. A typical example of p53, for instance, or survivin antigens. These molecules cannot be safety downregulated without severely affecting the ability of tumor cells to grow.
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00:04:33,000 | 5 | Immune response, any immune response, depends on two groups of cells. One is the cells which present antigen. We call them antigen-presenting cells. And another one, the cells which respond to those antigens, are -- and we call them effector cells. And we will discuss briefly both these groups of cells.

00:04:57,000 | 5 | Antigen-presenting cells can be arbitrarily divided on three major groups. One is so-called conditional antigen-presenting cells, the cells which are not designed to be antigen presenting, and very rarely perform that function. However, under certain circumstances, they can, especially in the situation of injury. In lung injury, for instance, is one example of it. This is endothelial cells, epithelial cells and T cells. These cells are very ineffective in antigen presentation, and as a result, they often not -- not induce T cell response, but rather tolerize the immune system.

00:05:36,000 | 5 | Semi-professional antigen-presenting cells. It's a group of cells which, although not designed to do -- to do the presentation, however they are very well equipped to do so. It's B cells and macrophages. They need to be activated in order to perform their function. They are serious, powerful cells which we take into account when we develop a therapeutic strategy.

00:06:00,000 | 5 | And the last group of professional antigen-presenting cells are basically dendritic cells. It's the cells which -- the same lineage as macrophages, but more specialized in antigen presentations. These cells are most powerful in antigen presentations, and they are very important for the induction of antitumor immunity. These cells are actually used in quite a few different clinical trials to induce immune response against different tumors, lung cancer in particular.

00:06:32,000 | 6 | And this slide just illustrates this interaction between antigen-presenting cells and effector cells and the T cells. Antigen-presenting cells are generated in bone marrow by the effect of multiple different cytokines, and the receptor which is expressed on the cell surface of the bone mass stroma. And these cells, expanded, differentiated, and come into the contact with the tumor, either necrotic or apoptotic. Tumor cells pick up these antigens, process them, present on the surface, get activated and stimulate T cell responses in the CD4 and CD8 cell responses. The end goal of all of this is to generate enough CD8 cytotoxic T cells able to recognize and kill tumors. And this is the ideal situation, which is, as we all know, not really happening in patients. And that's our goal, how to make it work.

00:07:34,000 | 7 | Dendritic cells are that powerful because of the unique function of its features. And this slide just briefly shows you what exactly we mean by these features. So the dendritic cells has a fantastic intracellular machinery to process antigens. They are very efficient in antigen presentation due to expression of the variety of surface molecules. They have a high potential of T cell stimulation. These cells can be expanded in ex vivo, and that was used in different clinical applications. And therefore, it's considered one of the best adjuvant for immune responses at this moment.

00:08:16,000 | 8 | So, the second arm of the immune response is effector cells. And of course, these effector cells can be subdivided into three major groups. It's the CD4 T cells, which play a helper role to support cytotoxic T cells. They also actually produce interferon gamma, which may be very helpful in antitumor activity, as well. Cytotoxic T cells, CTLs, are the major cells which recognize tumors through the antigen expressed by MHC class I. And natural killer cells, or NK cells, recognize tumor through the non-MHC class I molecules, and they also kill targets which are coated with immunoglobulin. We will talk about that in a couple of slides, a little bit later.
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<td>So how the CTLs or NK cells kill tumors? There are several mechanisms. The most important one is considered as the so-called perforin granzyme B. So granzyme B is produced by T cells, CTCLs or NK. And it's released upon activation. So if the T cells interact with appropriate antigen in the context of MHC class I, it's -- they will get activated and release granzyme B. They also -- NK cells will recognize their receptor on the surface of tumors. In absence of MHC class I, those NK cells also start releasing granzyme B. There are several other granzymes, but granzyme B is most effective and most powerful.</td>
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<td>So this is the enzyme which are basically getting inside the cells, causing apoptosis. And -- but in order to get inside the cells, this granzyme B had to be helped by another protein, another enzyme called perforin, which make membranes penetrable for the granzyme B.</td>
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<td>The second mechanism of killing is a little bit less powerful, but also important, is the -- through the so-called FAS, FAS ligand interaction. It's a death receptor expressed on the surface on the tumor cells, and the ligand expressed on the cytotoxic T cells, so NK cells.</td>
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<td>And the third mechanism of killing, it's also actually for FAS ligand -- a FAS death receptor, the end result of -- is activation of caspases and killing all the cells through the apoptosis.</td>
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<td>And the third mechanism, which is not shown on this particular slide, but also important is interferon gamma-mediated mechanism of killing. Interferon gamma by itself can trigger apoptosis in large amounts. A large amount of interferon gamma can trigger apoptosis and cause cell death.</td>
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<td>Immune response, which -- believed to be developed in certain stages by tumors, is really ineffective. So there's many different theories why immune system is not taking out tumors right away. There are -- a very strong opinion, which is supported by studies and shared by a significant number of investigators, that there is an early tolerance associated with early event of tumor development. So basically, the lytic cells, which are not activated in the conditions of normal non-inflamed tumor, they basically tolerate the immune system and not allow the tumor to get recognized.</td>
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<td>There is a very -- equally large, and maybe even bigger group of researchers which believe that there is really not a tolerance issue but rather ignorance, basically meaning that tumors is not sufficient numbers at the early stages to get -- be visible by -- to get visible by immune system, and therefore are not recognizable by cytotoxic T cells.</td>
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<td>Whatever the reason for the initial development of tumor, obviously, tumors develop not because of failure of immune system, except situations with immunocompromised patients, which we will discuss a little bit later. The main reason for the T -- the tumor to develop is, of course, molecular events associated with different abnormalities in genome. However, in the initial stage, regardless, whether it's a reason of tolerance or ignorance, the tumor developed.</td>
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<td>But then there is a stage of equilibrium, and that stage of equilibrium, which is proposed by Hope Schreiber from Washington University in St. Louis suggests that there is a compromise between the ability of NK and CD8 TCTLs to kill tumors and the tumor growth. So -- and that equilibrium may last for many, many years. And then, due to some reasons, which they're quite extensive, and we don't have time to discuss them at length, there is a shift in the balance. And the immunosuppressive factors, which is always present and produced by tumors, they overcome ability immune system to control. So that's called immune escape. So therefore there's -- a large effort is placed on the ability of tumor cells to escape immune system.</td>
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A couple words about B cells, because B cells, obviously, is a very important part of immune system, and especially in viral and bacterial infection. It's also important for tumors, as well. Maybe not in the way to control immune system, because there's enough evidence suggesting that antibodies and B cells do not really -- directly responsible in immune surveillance or control tumor progression.

However, they are important as a therapeutic tool, and it's also important to notice that the recent years, there's enough evidence indicating the B cells themselves playing a very important negative role in regulation of immune system. So basically, they support tumor escape from immune system recognition, is their work as a negative factor.

The B cell response requires help from CD4 T cells, which we already discussed briefly, and activated B cells undergo clonal expansion and become plasma cells, which produce immunoglobulin M, or IgM, which under the -- after the isotope switching become high-affinity immunoglobulin G, or IgG, which then can be used to target tumors. As we know, antibodies are -- play a very important role in triggering receptor by binding mechanisms, and then they're very important in elimination of tumors, as it was shown, is quite -- quite a few different antibodies targeting lymphoma in breast cancer.

The mechanism of killing induces several different paths. This slide illustrates the interaction between CD4 T cells and B cells to stimulate the production of antibodies.

And this slide basically showing that -- how exactly antibody can kill tumor through the complement-dependent cytotoxicity or, as many people demonstrate, maybe the main factor is so-called antibody-dependent cell-mediated cytotoxicity, or, as we call it, ADCC, which -- mediated by several cells, among them natural killer cells, NK cells, which we already discussed, and also granulocytes and neutrophils. They are actually very powerful as ADCC mediators. So antibodies, recognition of tumors has come -- work in concert with those cells able to kill tumor cells.

So we talk about immune escape quite extensively, and really, here, this slide just recapitulated those potential mechanisms of immune escape, which include loss of MHC class I, therefore the cells cannot be recognized by CTLs. Loss of tumor antigens, and that's what I mentioned to you, how important the adequate selection of antigen you're targeting, because if the loss of tumor antigen is not really that important, like in melanoma, for the cell survival, then cell -- tumor cells will be happy to lose the antigen and escape recognition.

A very important mechanism which is now widely appreciated is the inability of T cells to penetrate tumor stroma. So basically, we can transfer T cells either after adoptive -- expansion ex vivo and adoptive T cell transfer. We can create a very powerful new vaccine, but the T cells simply cannot get to the tumor. So that's very important to disrupt that tumor stroma, and that's basically a foundation of so-called combined or combinatorial approach to the cancer treatment, which will be discussed in different lectures.

Defective function of professional antigen-presenting cells, primarily dendritic cells, is a critical element of this problem, because without that it's very difficult to expect that immune system will be developed properly against tumor, and that -- that's what's really happening.

Immunosuppressive cells, they're called either T cells or myeloid cells, the T cells, as regulatory T cells, or myeloid cells are -- myeloid-derived suppressor cells, so MDSC, they basically are two major groups of cells, which through the multiple different mechanisms, suppress T cell function. They are both expanded in different types of cancers, and interestingly enough, in some type of cancer, regulatory T cells are a primary source of immune suppression. In others, MDSC. So it's a very interesting interplay between these two groups of cells, which needs to be explored in the future.
Inhibitory cytokines and molecules, that's another very important part of it, like molecules associated with B7-H1, B7-H4, which are targeted by molecules like PD-1, PD -- having so much promise these days in treatment of lung cancer. Those molecules also are critically important for induction of immune -- immune suppression.

The myeloid compartment, which we already briefly mentioned, is really affected dramatically in cancer, and lung cancer in particular. And what the result of this is that dendritic cells, which are normally produced in healthy individuals, they have a difficult time to differentiate, and instead the patients have a much higher level of so-called immature DCs, dendritic cells, which are not getting through the cell -- process of differentiation, and therefore are not effective in induction of immune responses. But also, they produce a lot of those immature myeloid cells, which now we call MDSC, which very powerful immune suppressive ability.

And this slide basically illustrated how many different mechanisms those MDSCs can employ to suppress different aspects of T cell function. In addition, they also can trigger expansion of regulatory T cells, and therefore just kind of trigger this vicious circle, where they -- each factor amplifies each other to get a suppression underway.

Regulatory T cells, obviously, are a very main player in the induction of immune suppression. They are considered as -- in two different varieties of the cells, the ones which are generated in time as immune suppressor cells, and another one which are converted on the periphery from normal cells. There are now a quite clear understanding what are the mechanism of this conversion and how important that different cytokines in this conversion, like TGF-B, for instance, and in different specific receptor molecules. We will not go to this detail, too much specific details. We don't have time on that for that. But suffice to say that these cells really can be very powerful players in inhibition of immune responses in cancer.

In addition to those two major groups of cells, MDSC and T regs as I mentioned to you, there are plenty of soluble factors which play a very important role in lung cancer specifically, in particular. This slide illustrates the very important role of one of them, which has been studied quite extensively. It's indoleamine-pyrrole 2,3-dioxygenase, or IDO. And the factor which -- basically the role of this IDO is a single-chain oxidoreductase, which participates in tryptophan catabolism and basically making kynurenine from tryptophan, and which is important for the synthesis of nicotine, adenine, the nucleotide.

What IDO does in high expression, if it's too much IDO, it prevents tryptophan to support CD4 expansion. It, also through the kynurenine, blocks the function of CD4 T cells. And you can see here that this is described -- this slide describes these steps on this pathway, and how the immune system can be boosted by the elimination of IDO suppressive cells.

Elimination of regulatory T cells, or FoxP3-positive cells, is also another very important mechanism how one can induce immune response, and these therapeutic strategies has been pursued in differently varieties with some successes. Again, this study's in very early stages of development, so the clinical significance is still unclear.

We were talking about the mechanism of tumor development, and I mentioned to -- that in non-compromised patients, the tumor developed because of multiple different molecular mechanisms not associated with the immune system. But at the later stages of tumor development, the tumor becomes visible for immune system, and where that -- those immune suppressive mechanisms kicked in. However, if the immune system is important, then immunocompromised patients, the incidence of cancer should be much, much higher.
And this slide, a couple of slides which I showed you know, basically illustrated that fact, which we all appreciate now, that the incidence of cancer, different types of cancer in immunocompromised patients, HIV/AIDS patients and transplant recipients, really dramatically increased. So this is the list of the different studies showing this dramatic increase of standardized incidence ratio.

And the next slide showing this different ratio with -- of cancer with people with only HIV alone.

This is a metaanalysis of different types of cancer and the incidence lung cancers, I'm sorry, in people with HIV and transplant recipients. All of those data are basically showing the same thing, that if you eliminate control from immune system, the incidence of cancer is substantially increased.

One of the main findings in recent clinical trials and recent observations is that if patients have a high level of infiltration by T cells have a much better prognosis. So the T cell infiltration of tumors is really associated with a favorable prognosis. This is kind of -- It may seem like a trivial observation, but in reality it took many decades of studies to produce convincing evidence of this, because these data clearly indicated how important generation of potent immune response to achieve better clinical outcome.

And it's important to point out that we compile only the data on -- associated with non-small cell lung cancer here, and this particular slide only references those papers. But there are now a large body of evidence, many other types of cancer where the T cell infiltration and the different type of T cells is closely correlated with better prognosis, outcome of chemotherapy and many other parameters.

So there are now a very -- really concerted effort to produce those immunophenotyping parameters as the prognostic markers which -- to supplement commonly used pathological markers of -- to better define prognostic values of this parameter to test and make it clinical useful.

So in my presentation today, I described to you the multitude of different factors which influence immune response in cancer. And we talked about immune escape, we talked about the possibility to recognize and kill tumors. Obviously, we would like to know whether there is a clinical significance of all of this, direct evidence. In the recent years, we have very clear evidence -- there is a direct evidence of importance of immune system in cancer. And they come in a different variety of data.

And one of them is correlation between tumor infiltrates with lymphocytes on the T cells specifically, and clinical prognosis. There are a large amount of data right now obtained from the different trials in patients with different diseases. Here we compile only the ones which are concerned directly with lung cancer.

The message from all of these trials is that now you can link the T cell infiltration of the tumors with a better outcome of the disease and response to chemotherapy and other different clinical parameters. Moreover, there is a now concerted effort by researchers to put together the specific criteria which can be used for the prognosis of the disease using these immunologically relevant data.

So right now we have really a very exciting time when we have a very high expectation that regulation, manipulation of immune system will be able to substantially improve the outcome of lung cancer. And I'm -- I know that you will hear more specific examples of that in the different lectures. But for me, I have to thank you for your attention, and I hope my presentation was useful. Thank you.