Dr. Demetri: In answer to your first question, there is a ton of work that has been performed in cell-cycle inhibitors of various types and it has been remarkably fruitless. However, that also may be because it was not performed in the right patient population. For some reason, the minute they go into hormone-receptor–positive breast cancer, CDK4 inhibitors have breakthrough designations, which is extraordinary. Part of the problem again is matching the drug with the right patient, validating the targets, and figuring out how to hit the target effectively. We have to be careful not to jump to conclusions. We often say the drug failed, but, instead, it may just be the wrong patient.

To your second question about how many drugs may be needed for personalized cancer treatment: who would have guessed that the same drug would treat leukemia and solid sarcoma? This again suggests that we must bin tumors by mechanism and we still do not know how to do that well. I suspect you will be binning things by mechanism of glomerulonephritis, perhaps by mechanisms that also apply to interstitial pulmonary fibrosis (IPF). The world has just had a couple of really great targeted drugs approved for IPF. IPF is worse than most cancers and IPF could have mechanisms that really affect what you are working on in nephrology.

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Genetics of Familial FSGS

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I am going to talk about familial focal segmental glomerulosclerosis (FSGS) today. As everyone realizes, FSGS is a histopathologic entity, a pattern of injury that is seen on a kidney biopsy. Nephrotic syndrome is just that, a syndrome. Hypertensive kidney disease also shares features with FSGS. There is considerable overlap between all of these entities. Even though these are just names, I think the fact that we do not have really good ways for the clinicians and the pathologists and patients to talk to each other about these entities does cause some problems. Nevertheless, today we will focus on familial FSGS.

As a historical note, while I was still in training, I came across a family with inherited kidney disease in Oklahoma, in a report published in 1998.[1] I flew out to a little town outside of Tulsa, Oklahoma, and spent 2 days rounding up members of this family and taking their blood pressures, talking to them, and obtaining urine and blood samples.

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They had a dominant inheritance of a phenotype that was characterized by proteinuria. A few individuals had biopsy-proven FSGS. We started to perform genetic studies to try to identify the underlying gene and also scour the world for other similar families. The advantage of this kind of approach is that one does not need to worry about whether the amount of proteinuria or the histology looks exactly the same from person to person. It alleviates the concern about having a good way to classify patients diagnostically.

We therefore performed what at the time were fairly laborious genetic studies, and ultimately this led us to the identification of mutations in the α-actinin-4 gene as the cause of the disease in two families with FSGS, as well as several others.[2-4] All of the mutations that segregated with disease in these families were in the actin-binding domain of α-actinin-4, matching well with the best described function of α-actinin-4, which is to cross-link actin filaments.[2-4]

Indeed, the actin-binding domains associated with FSGS-causing mutations have a much greater affinity to actin filaments than wild type α-actinin-4. I therefore suggest that these mutations are causing a dominant effect by a gain-of-function change in their behavior. This led us to start thinking about what may be going on structurally and we ultimately found that the actin-binding domain could exist as both a closed and an open confirmation.[5,6] We therefore concluded that mutations that were associated with kidney disease and led to increased actin binding interfered with the transition between the closed and open states.[5,6]

We hypothesized further that the presence of disease-associated mutations may alter the behavior of the podocyte cytoskeleton. We have been very interested in what this means for the biophysical properties of the podocyte and how we can connect in vitro cytoskeletal behavior with how the podocyte actually behaves. We are making slow progress toward this.

If we create reconstituted α-actinin-4/actin networks and perform rheologic studies, in work performed in collaboration with colleagues from the Physics Department at Harvard, we note that when we apply increasing stress to wild-type α-actinin-4/actin networks, we find increasing stiffness of the network until finally it breaks at a certain level of stress.

With these disease-associated mutations, this occurs at a much lower level of stress, resulting in excess stiffening and breaking of the actin cytoskeleton. When the actin-binding domain is removed from α-actinin-4, the cytoskeleton networks do not stiffen first, they just break all of a sudden.[7] The big question, and what we actively are trying to understand now using a variety of biophysical methods, is to see if this behavior translates into altered biophysical behavior of cells in culture, and we hope eventually also in cells in vivo.

This observation raises another question, which I think has therapeutic implications. Even though these mutations are an exceptionally rare cause of kidney disease, I think the hope is that this will help us understand more common forms of disease, not just diseases caused by point mutations in α-actinin-4. From an evolutionary point of view, although the actin-binding domain of α-actinin-4 normally is hidden, it is highly conserved in evolution. Even Drosophila α-actinins have a hidden actin-binding domain. Why would this exist if all that it does is cause kidney disease when it is exposed to actin through gain-of-function mutations? The answer is that it must have other functions.

The notion is that perhaps there are physiologic signals in the cell that are important in controlling whether α-actinin-4 has a closed or open confirmation and essentially control the strength of its interactions with actin. This is something we have been exploring using a variety of methods. For example, we are trying to understand whether actin binding is controlled through post-translational modifications of α-actinin-4.

This is a work in progress, but I hope it shows that understanding rare forms of disease can lead us to make interesting hypotheses in understanding more generalized mechanisms of disease.

Mutations in INF2 cause familial FSGS.[8] This is based on work performed by Elizabeth Brown in my laboratory and now replicated by many groups. There are a large number of independent mutations in a protein called INF2 that progressive chronic kidney disease with proteinuria, typically FSGS on kidney biopsy, but generally characterized by subnephrotic ranges of proteinuria.[8] A variety of mutations now have been identified in INF2 and they all cluster in the same domain of the protein, a region called the diaphanous inhibitory domain (DID).

What is INF2? It is a member of the formin subfamily of proteins. These proteins regulate the actin cytoskeleton, but in a very different way than α-actinin-4 does.

INF2 and other members of the formin family sit on the so-called barbed end of an actin filament and they stimulate oligomerization of actin filaments. INF2 exists as a dimer, as does α-actinin-4. The business end of the molecule that stimulates actin polymerization is near the C terminus of the molecule. An interaction between the domain DID and the diaphanous autoregulatory domain inhibits the actin regulatory function of the INF2 protein.

All the mutations that cause human disease are in the DID, where they result in the loss of the normal inhibitory effect of INF2 on actin.

However, we do not think that is the sole reason for disease. The end terminus, the DID region of INF2, also interacts with other formin family members. Formin family members are downstream effectors of the Rho family of small guanosine triphosphatases