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NEWS

Biological and Pharmacological Effects of Estrogen and SERMs on Bone

Distinguished presenter and panelists discussed mechanisms of action and key clinical issues during the second IBMS BoneKEy Online Forum

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The story of how selective estrogen receptor modulators (SERMs) act at the molecular level to produce responses in target tissues is far more complicated – and surprising – than experts envisioned even just five years ago. Such was the case made by Donald McDonnell in his presentation for the second IBMS BoneKEy Online Forum, "Biological and Pharmacological Effects of Estrogen and SERMs on Bone," which took place in late April. An esteemed panel of bone experts followed the main presentation on mechanisms of SERM action with a discussion of how the new perspective on SERMs illustrated by Dr. McDonnell might impact the bone field's understanding of a variety of important clinical issues, including differences between SERMs and the role of these drugs in male osteoporosis.

Fundamental Tenets of SERM action

After reviewing the current understanding of estrogen's genomic and non-genomic activities (Fig. 1), Dr. McDonnell, a professor of molecular cancer biology in the department of pharmacology and cancer biology at Duke University Medical School in Durham, North Carolina turned to the underlying science that explains the mechanism of action of SERMs. These agents bind to the estrogen receptor and in doing so alter the conformation of the receptor in a way that depends upon the characteristics of each particular SERM; this was the first unexpected finding. "Pharmacologically, this was a surprise because everyone thought that there was either an 'on' or 'off' form [of the receptor], but the overall shape of the receptor was different and determined by the nature of the bound ligand," Dr. McDonnell told the international BoneKEy audience listening to the webinar.

Differences in the conformation of the receptor are important because they determine which cofactors – proteins that activate or in some cases repress transcription – bind to the receptor. The number of these co-factors was a second surprise. "Originally it was thought that there would be one or two different cofactors that the receptor can interact with, but there are probably over 300 that the estrogen receptor can actually touch during various points of its life, when you consider all tissues," Dr. McDonnell emphasized. The nature of the co-factors that bind to the receptor is significant because this will impact the biological activity of the receptor-cofactor complex. Dr. McDonnell's own research focuses on just this area: he and his colleagues are trying to determine which complexes account for the biological activities of SERMs in different tissues (Fig. 2). Ultimately, because of these differences in receptor shape and co-factor recruitment, another fundamental tenet underlying current thinking in the field is that not all estrogens/SERMs are the same. Considered together, these new insights have resulted in a new general view of nuclear receptor pharmacology (Fig. 3).

While knowledge of this quite complicated underlying science guides the pharmaceutical development of SERMs, Dr. McDonnell stressed that naturally-occurring molecules in the body, beyond estrogen produced by the ovaries, also utilize this biology, and he described the characteristics of 27-hydroxycholesterol (27HC) to prove

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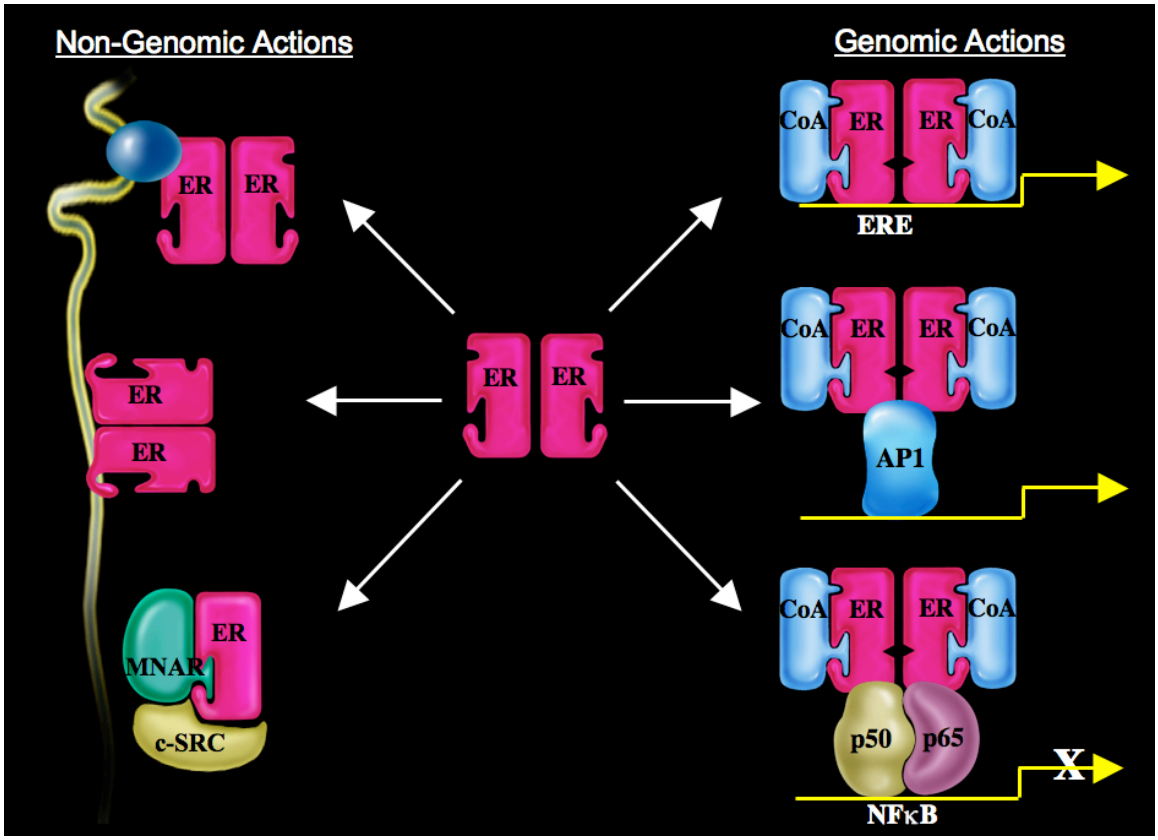

Fig. 1. The potential fates of agonist-activated estrogen receptor (ER). The ER can interact directly with specific DNA sequences as well as indirectly through binding to proteins such as activator protein (AP) 1 to activate transcription (top and middle right-hand side). In addition, the ER can also interact with other proteins to repress transcription, for instance through the nuclear factor κB (NF-κB) pathway (lower right). Estrogen also has non-genomic actions (left-hand side). These actions can be initiated either in the membrane directly or through the interaction of the ER with other signaling molecules in the cytoplasm of cells. CoA = coactivator; MNAR = modulator of nongenomic action of estrogen receptor.

his point. Indeed, like the SERMs developed by pharmaceutical companies, 27HC, which is a metabolite of cholesterol (Fig. 4) and is present in the blood serum at micromolar concentrations, binds to the estrogen receptor and upon binding alters its shape. This then allows the 27HC-estrogen receptor complex to interact with unique co-factors resulting in the activation or suppression of transcription of estrogen-receptor target genes. Research findings have also revealed that the degree to which 27HC acts as an agonist or antagonist differs between cells and between genes in the same cells.

Furthermore, experiments also indicate that 27HC's behavior as a naturally-occurring SERM has relevance for bone, where 27HC is produced by macrophages. Indeed, Dr. McDonnell and his colleagues have studied knockout mice missing an enzyme, called CYP7B1, which degrades 27HC. These mice, which have elevated levels of 27HC, exhibit significant decreases in bone mineral density at the femur, lumbar spine, and in cortical bone. (Since *in vivo* studies of 27HC are only in the early stages, whether 27HC plays a role in postmenopausal women is unknown).

The gist of all of these findings, according to Dr. McDonnell, is that a more expansive view of SERMs is required. “27HC is a molecule that circulates in the body, is produced not by the ovaries but by macrophages, and can also impact the estrogen receptor. This suggests that nature has used the SERM concept before.” Ultimately, Dr. McDonnell asserted, “we need to expand our view of what an estrogen is, beyond just the ovarian steroid.”

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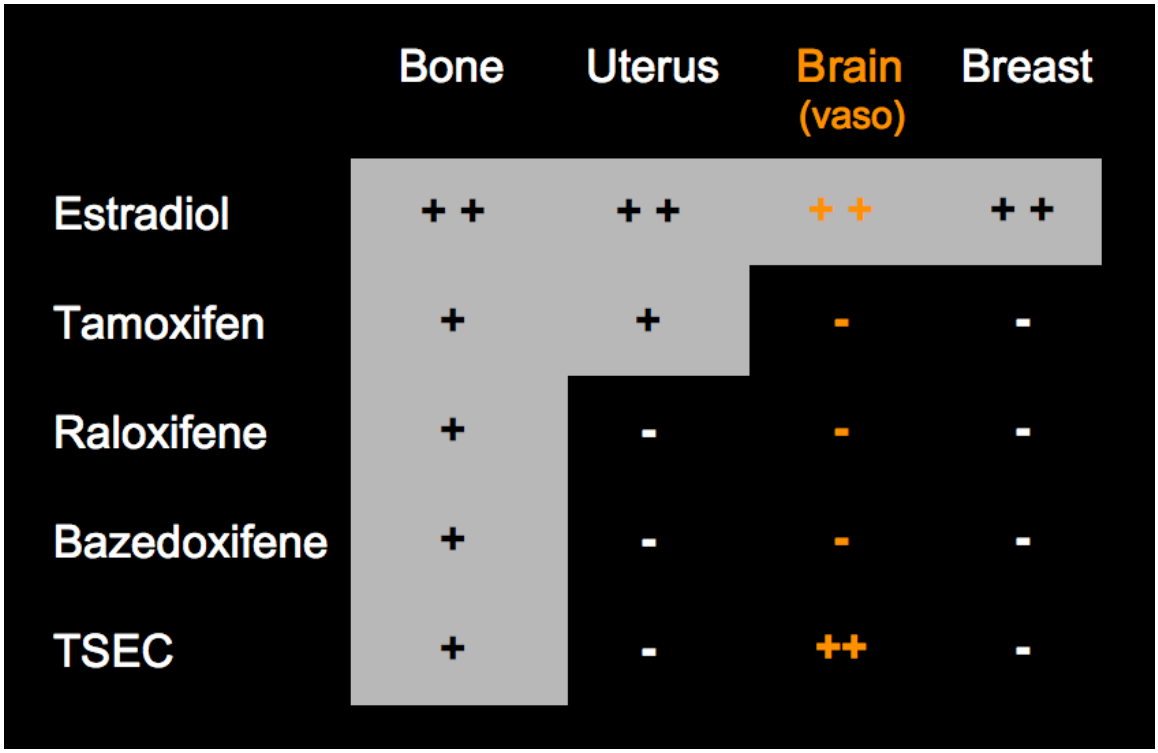

Fig. 2. The effects of SERMs/ER modulators in various tissues. + = effect; - = no effect; TSEC = tissue selective estrogen complex; VMS = vasomotor symptoms.

In addition, he noted that it is important to recognize, whenever SERMs are administered, that there are molecules, like 27HC, that may augment or weaken the activity of these agents.

Another Receptor

As interesting as the research with 27HC is, perhaps the biggest surprise of all is that the estrogen receptor is not the only game in town: some of the actions of SERMs appear to be mediated independently of the estrogen receptor through another receptor, the aryl hydrocarbon receptor. Small interfering RNA (siRNA) experiments in which the aryl hydrocarbon receptor is knocked out have shown this to be the case for the SERM tamoxifen. Indeed, these studies show that, in the absence of the aryl hydrocarbon receptor, tamoxifen loses its ability to regulate the transcription of genes that it normally regulates when the receptor is present. In fact, Dr. McDonnell and his colleagues have shown that a large number of the genes that tamoxifen is currently known to regulate are, after all, regulated through the aryl hydrocarbon receptor, and not the estrogen receptor.

Additional experiments have revealed that these aryl hydrocarbon receptor-dependent pathways are actually important in explaining the ability of SERMs to affect osteoclastogenesis. For instance, when the estrogen receptor is knocked out with siRNA, tamoxifen's ability to suppress osteoclastogenesis, as measured by an *in vitro* osteoclastogenesis assay, is not weakened. However, when the aryl hydrocarbon receptor is knocked out, while estrogen and raloxifene still suppress osteoclastogenesis, tamoxifen's ability to do so is blocked. “This was an eye-opener experiment because what it says is that for too long, we have been focused on the estrogen receptor as the primary and only target of SERM action,” Dr. McDonnell said.

Also emphasizing the importance for bone of estrogen/SERM effects on osteoclastogenesis was panelist Stavros Manolagas, who noted that effects on osteoclastogenesis are observed when the estrogen receptor is knocked out from early

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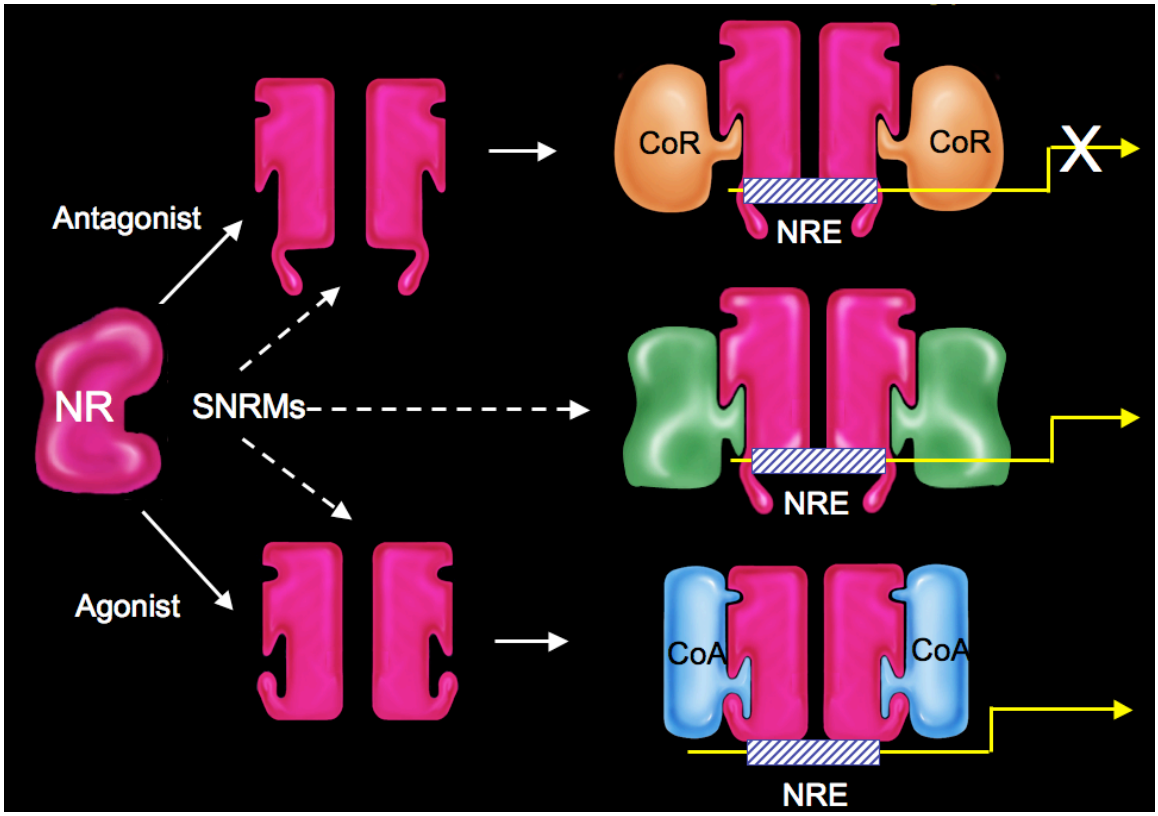

Fig. 3. An updated model of nuclear receptor (NR) pharmacology. Agonists bind to the receptor, alter the receptor's shape, and in general allow for the recruitment of coactivators (CoA) to activate transcription. Upon binding to the receptor, antagonists also alter the receptor's shape, resulting in the recruitment of corepressors (CoR) to repress transcription. Selective nuclear receptor modulators (SNRMs) allow the receptor to take a form that is intermediate between the inactive and active states. NRE = nuclear response element.

progenitor cells. In addition to effects on the bone-resorbing cells, Dr. Manolagas also emphasized the impact of estrogens/SERMs on other cell types in bone. “Overwhelming evidence indicates that estrogens not only affect chondrocytes and have control of the growth and differentiation of the growth plate, but they also affect the viability of osteoblasts and osteocytes, and I think this is a very important mechanism,” said Dr. Manolagas, a professor of medicine at the University of Arkansas for Medical Sciences in Little Rock. However, whether different SERMs may have different effects on osteoblasts is not known.

Clinical Questions

Can this new perspective from the basic science side on the molecular pathways, and the receptors involved in those pathways, account for the varying clinical activities of SERMs, especially newly developed ones like bazedoxifene and lasofoxifene? “It's extremely difficult to draw correlations between the complex biology that has been described and the clinical effects of SERMs,” according to panelist and osteoporosis expert Steven Cummings, director of the San Francisco Coordinating Center at the California Pacific Medical Center Research Institute. As an example, Dr. Cummings pointed to a surprising characteristic, documented in a recent clinical trial, of lasofoxifene: it was found to have an effect on C-reactive protein, a marker of inflammation, and appeared to decrease inflammation as well as the risk of cardiovascular disease, coronary heart disease, and stroke. This surprise, he said, could not have been predicted from the mechanisms that Dr. McDonnell had described.

Regarding lasofoxifene, of particular note is that it has been found to decrease the

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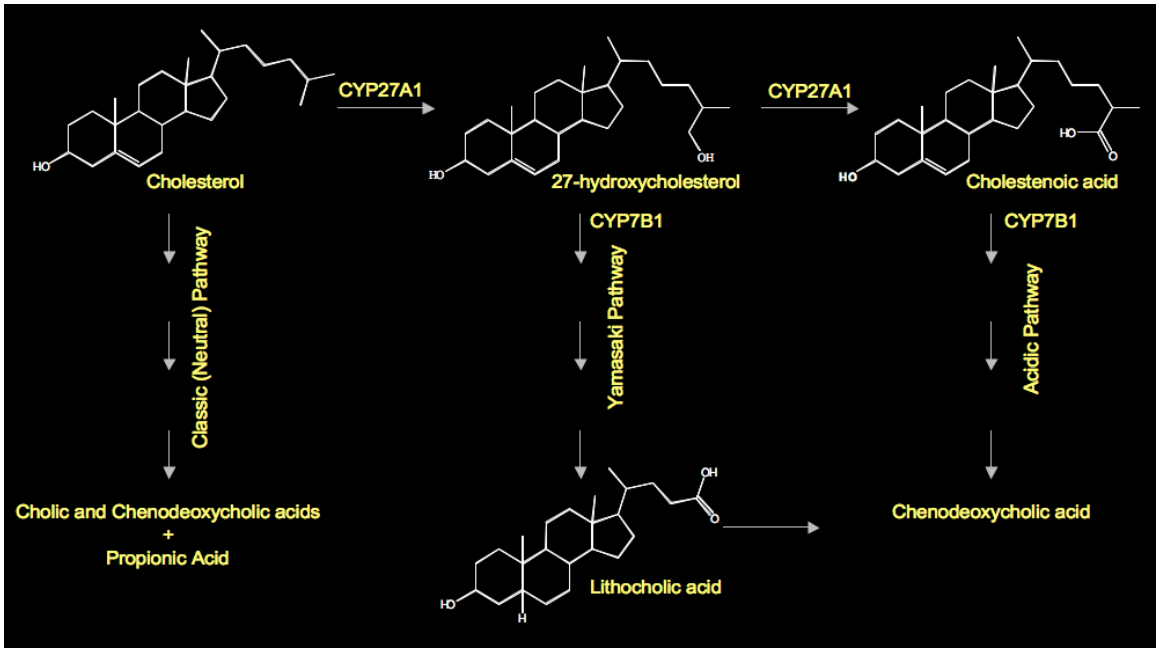

Fig. 4. 27-hydroxycholesterol (27-HC): a product of bile acid metabolism. 27HC is converted from cholesterol by the enzyme CYP27A1 and is degraded by the enzyme CYP7B1.

overall risk of non-vertebral fractures, while previously it was found that raloxifene did not and another new SERM, bazedoxifene, may do so only in a subset of higher risk patients; this is another example of a clinical difference between SERMs that remains unexplained. Dr. Cummings noted that the differences between the two drugs' effect on non-vertebral fractures is hard to explain by pointing to bone density, since both drugs improve this measure by only about 3%. “There may be other mechanisms of action, besides the simple effects of bone density, on other aspects of bone that we don't yet understand,” Dr. Cummings explained.

In addition to discussing clinical differences between SERMs, the panel also discussed another key clinical question, namely the strength of the evidence to support a role of sex steroids for bone health in men. “It's clear that androgens are important for the male skeleton. Some of the effects of androgens on bone are probably mediated directly through the androgen receptor, however, recent data indicate that some of the effects of androgens on bone are mediated indirectly via aromatization to estradiol followed by activation of estrogen receptors,” explained Claes Ohlsson, an expert in this area at the University of Gothenburg in Gothenburg, Sweden. Dr. Ohlsson noted that a major finding in support of the contention that estrogens play an important part in bone health in men is that “serum estradiol levels are in general more strongly associated with bone mineral density, bone turnover markers, and bone loss than testosterone levels in adult men.” However, Dr. Ohlsson added that, going beyond bone density, the evidence for the association between sex steroids and fractures in prospective studies is inconsistent. Some studies have reported that neither serum estradiol nor testosterone predicted fracture risk. On the other hand, one study found that low serum estradiol, but not testosterone, predicted fracture risk, while another recent study found evidence for the opposite conclusion, reporting that low testosterone, but not serum estradiol, predicted fracture risk in men. Dr. Ohlsson suspects these contradictory findings could be due to study design issues – studies have been underpowered, including few incident fractures – and also because of the immunoassay-based techniques used to measure baseline sex steroid levels.

To overcome these issues, Dr. Ohlsson and his colleagues recently studied men from the MrOS Sweden study, using mass spectometry to measure baseline levels of sex steroids. “We found that both serum

estradiol levels and testosterone levels were inversely associated with fracture risk when analyzed separately. However, in multivariate analyses, serum estradiol, but not testosterone, was an independent predictor of fracture risk in these elderly men." Dr. Ohlsson also pointed to evidence supporting a threshold effect of estradiol for the male skeleton.

Considering this basic framework, then, regarding the effects of testosterone that are mediated through estradiol and the estrogen receptor, a natural question to ask is whether SERMs could be used in the treatment of male osteoporosis. Panelist Luigi Gennari of the University of Siena, in Siena, Italy explained to the BoneKEy audience that preclinical investigations with SERMs, such as lasofoxifene, suggest the drugs may prevent bone loss induced by aging or orchidectomy in males. However, the primary concern is the potential for adverse effects on other estrogen targets such as the gonads, prostate gland and cardiovascular system; only short-term data are available now in this regard. Meanwhile, ongoing clinical studies suggest that SERMs may have a place in male osteoporosis therapies, but with certain limitations. For instance, phase 2 clinical trials with raloxifene suggest that this SERM might have positive effects on bone, "but only in male patients with estradiol levels below a certain threshold that is quite similar to the threshold that has been observed by investigators in cross-sectional and longitudinal studies. Above these thresholds, raloxifene is not good for bone, as it will cause an increase in bone resorption [by competing with endogenous estradiol]," Dr. Gennari said. At this early stage, Dr. Gennari believes that SERMs could eventually play a significant role in preventing bone loss particularly in vulnerable men, such as those receiving androgen deprivation treatment and in aging men with low estradiol levels.

Final Questions

A final matter taken up by the panel was a controversial one: what is the effect of estrogens on the periosteum? The panel agreed that, while estrogen clearly has stimulating effects on the periosteum during growth and development, whether there are effects beyond puberty is unknown. "I'm afraid that we cannot really comment with any certainty on whether estrogens have effects on the periosteum beyond the stage of growth. This is clearly an issue that needs major investigation" said Dr. Manolagas. A final question that Dr. McDonnell also addressed was whether SERMs have differential effects on estrogen receptor-α and estrogen receptor-β. He noted that while there are indeed differences between SERMs in this regard, what is unclear is how such differences translate to responses in the body. He predicts that, now that the field is beginning to have compounds that are receptor-specific, the next 18 months or so should provide some insight into this issue.

The future then, is full of questions, on both the basic science and clinical sides, but it is undeniable that researchers have a much more solid understanding of estrogens/SERMs than they did just half a decade ago. "I think we've moved a long way in our understanding of SERMs. Five years ago, we thought it was rather simple and believed that SERMs were partial agonists, but I think that now we understand the complexities of the molecular pharmacology of the estrogen receptor and we realize that SERM action is quite complex," Dr. McDonnell concluded his talk, also noting that this new understanding will certainly have strong implications for pharmaceutical development. "Out of these complexities, new drugs are going to emerge."