Table 1. Tumor Incidence in In Vivo Tumor Initiation Assay

Injected Cells From PyMT Mice	Tumor Incidence in Mice	
	Wild Type (n = 10)	MTDH Knockout (n = 10)
Unsorted pMECs, No.		
100	1	0
500	3	0
2000	10	4
P value	< .001	
	Wild Type (n = 12)	MTDH Knockout (n = 12)
Sorted luminal cells, No.		
100	1	0
1000	5	0
10000	7	1
P value	< .0001	

MTDH, metadherin; pMEC, primary mammary epithelial cells; PyMT, mouse mammary tumor model with high rate of lung metastases (mouse polyomavirus middle-T antigen under the control of mouse mammary tumor virus).

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Adapted from *Cancer Cell*, Copyright 2014;26:92-105, Wan L et al. MTDH-SND1 Interaction Is Crucial for Expansion and Activity of Tumor-Initiating Cells in Diverse Oncogene- and Carcinogen-Induced Mammary Tumors. With permission from Elsevier.

expressed. These were cloned and overexpressed in tumor cells to determine which could drive lung metastases in a mouse model, leading to the identification of one gene, metadherin (MTDH), a poor prognosis marker and functional driver of breast cancer metastasis.

Knockout of MTDH does not affect embryonic or postnatal development in mice; in a model of a highly aggressive tumor, it slows tumor progression, reduces total tumor burden, and eliminates metastases. Tumor formation in MTDH knockouts in an in vivo model of tumor initiation is shown in Table 1.

Staphylococcal nuclease domain containing 1 (SND1) is associated with poor prognosis and interacts with MTDH. Knockdown of SND1 reduces lung metastases and sensitizes cells to chemotherapy-induced apoptosis [Blanco MA et al. *J Biol Chem*. 2011; Wan L et al. *Cancer Cell*. 2014]. Dr Kang's group has used crystal structural analysis to determine the sites of interaction between MTDH and SND1 [Guo F et al. *Cell Reports*. 2014]. Mutation at either 1 of 2 tryptophans eliminates the binding of MTDH to SND1 and the ability of MTDH to rescue tumor-initiating functions. The group is now screening small molecular compounds to interfere with the cooperative activity of MTDH and SND1 in tumor initiation.

Genes that confer survival advantage to primary tumor cells during tumor initiation may play crucial roles in metastasis via several mechanisms, including promotion of the development of tumor-initiating cells or by mediation of crosstalk between tumor cells and their environment. Therapeutic targeting of metastasis genes may have a far-reaching impact on the prevention and treatment of metastatic diseases.

Overcoming Tumor Diversity and Adaptability Key to Effective Therapies in BC

Written by Muriel Cunningham

While the current outlook for most patients with breast cancer (BC) is positive, challenges remain in finding the optimal therapies for the patients diagnosed with metastatic disease, according to Joan S. Brugge, PhD, Harvard Medical School, Boston, Massachusetts, USA, who shared her thoughts on the future treatment of these patients.

Advances in the genetic analysis of tumors have revealed astounding genetic diversity in BC. Multiple alterations are possible, with each patient having individual patterns of alterations. Questions that have emerged include how current therapies are affected by these variable alterations and whether therapies will need to be based on the genetic patterns of each patient. For example, research has shown that human epidermal growth factor receptor 2 (HER2)–targeted therapies are less effective in patients with amplified HER2 and PIK3CA alterations; adding a PIK3CA inhibitor may increase the effectiveness of HER2 therapies in patients with these combined alterations. Dr Brugge said that not all of the alterations influence sensitivity to therapy, and clinicians may be able to stratify patients into a still large but manageable number of subsets, which can be treated with different combinations.

Within each tumor, there may also be significant heterogeneity. Within a tumor, cells that are sensitive to a particular agent will be killed, leaving the surviving resistant cells. This can lead to variable success in the treatment of each tumor within a patient. Recent evidence suggests that a single residual cell can develop into a recurrent tumor. A patient with metastatic BC did not respond to standard chemotherapy and was found to have an activating PIK3CA mutation [Juric D et al. *Nature*. 2014]. The patient was treated with the PIK3CA-specific inhibitor BYL719 in a clinical trial. After an initial response, the patient developed resistance to the drug and died. Genetic sequencing of metastatic sites showed additional and different PTEN genetic alterations in nonresponding tumors. The fact that

these alterations were not present in the primary tumor suggests that a single cell mutated and was able to expand to produce a recurrent tumor in the presence of a drug. Dr Brugge noted the implication that every cell in a tumor must be deleted to prevent regression, which would present a serious treatment challenge.

The most effective tactics of the future will be those that disrupt the tumor ecosystem and block the ability of tumor cells to adapt. This will be a complicated approach requiring multiple agents. One potential strategy is to find the critical nodes of interaction within the tumor ecosystem and target them simultaneously to prevent adaptation. For example, the combination of hormone therapy, a stem cell–targeted inhibitor, a macrophage inhibitor, and a checkpoint inhibitor might prove to be effective. Taking out these nodes would cut off the support system of tumor cells, making them more vulnerable to destruction by the immune system. Another option is to target programs that are "downstream" from oncogenic pathways and pathways activated by microenvironment factors that coalesce to regulate cell survival in the context of cancer therapy, thus preventing "adaptation."

Highlights and Future Directions in AI Clinical Research

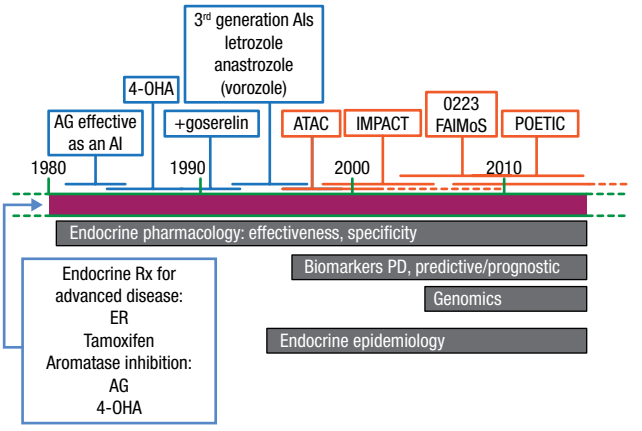
Written by Muriel Cunningham

Mitchell Dowsett, PhD, Royal Marsden Hospital, London, United Kingdom, gave a brief history of the development of aromatase inhibitors (AIs) and an overview of ongoing research seeking to further elucidate the role of hormones in breast cancer (BC). Developed in the 1990s, AIs are approved for the adjuvant treatment of early hormone receptor-positive BC in postmenopausal women. A timeline of AI development according to Dr Dowsett's lab is illustrated in Figure 1.

The availability of pharmacodynamic biomarkers helped to accelerate the development of AIs. Plasma estradiol assays with high sensitivity and specificity were particularly useful in letrozole and anastrozole research. According to Dr Dowsett, biomarker analyses of these agents demonstrated that the extreme suppression of estrogen levels would lead to clinical responses that no true phase 2 trials had been studied. It is estimated that this reduced the development time by 12 to 18 months.

In the ATAC trial [Baum M et al. *Lancet*. 2002], anastrozole was compared with tamoxifen and a combination of anastrozole plus tamoxifen as adjuvant therapy in >9000 patients with estrogen receptor (ER)-positive early BC. At 3 years, there was a significant improvement in the anastrozole group compared with the tamoxifen group (HR, 0.83; 95% CI, 0.71 to 0.96; $P = .013$). This benefit was shown to persist at 10-year follow up [Cuzick et al. *Lancet Oncol*. 2010].

Figure 1. Timeline of Aromatase Inhibitor Development



4-OHA, 4-hydroxyandrostenedione; AG, aminoglutethimide; AI, aromatase inhibitor; ER, estradiol; PD, pharmacodynamics.

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The IMPACT trial [Dowsett M et al. *Clin Cancer Res*. 2005] was similar to the ATAC protocol but biopsy specimens were taken before and at 2 and 12 weeks after treatment initiation. The change in the cellular proliferation marker Ki67 was significantly greater with AI treatment vs tamoxifen ($P = .004$ at 2 weeks and $P < .001$ at 12 weeks) and suggested that Ki67 may correlate with patient outcomes. To further investigate whether the pretreatment or 2-week Ki67 levels predict recurrence-free survival, the POETIC trial [Dowsett M et al. *J Natl Cancer Inst Monogr*. 2011] has randomized nearly 4500 patients with early stage ER-positive BC. Two-thirds of the patients were randomized to AI therapy before surgery, while the other patients received standard hormone therapy. Biopsies and Ki67 measurements were conducted at diagnosis and 2 weeks later at surgery.

Other research has shown that plasma levels of estradiol are correlated with the expression of estrogen-regulated genes in postmenopausal ER-positive BC [Dunbier AK et al. *J Clin Oncol* 2010]. To date, 3300 core biopsy pre- and post treatment samples have been obtained in the POETIC study. Whole genome expression was suppressed in 926 and increased in 401. Dr Dowsett commented that this provided the opportunity to try to identify patient-specific mechanisms of resistance. To see if the menstrual cycle can be utilized as a test of hormone sensitivity in premenopausal women, the MenCER study was designed and has completed recruitment. Seventy paired biopsies from participants have been obtained and analysis is ongoing.