Avant Diagnostics Highlights Published Clinical Utility Data for Theralink® mTOR Pathway Activation Data Predicting Tamoxifen® and Other Endocrine Treatment Response in Estrogen Receptor Positive (ER+) Breast Cancer

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- Two thirds of all breast cancer (BC) tumors are estrogen receptor positive (ER+)
- ER+ BC patients have mixed responses to endocrine therapy
- Research demonstrates mTOR pathway activation status prospectively predicts endocrine therapy treatment response

Avant Diagnostics, Inc. ("Avant") (OTC: AVDX), an oncology-focused healthcare technology company commercializing the proprietary Theralink® phospho-protein biomarker platform across multiple cancers, today highlighted independent published literature that provides critical validation for the clinical utility of the Company's proprietary Theralink® mTOR phosphoprotein assay in segmenting the estrogen receptor positive (ER+) breast cancer (BC) population by mTOR activation status. The data, published in multiple journals by leading independent research groups, demonstrates that ER+ BC patients whose tumors had highly activated mTOR pathway who were treated with anti-endocrine therapy such as Tamoxifen® are likely to exhibit treatment resistance, whereas patients whose tumors had low levels of mTOR pathway activation are likely to respond to treatment. Taken together, the data provides a compelling case for physicians treating ER+ BC patients to seek-out mTOR pathway activation status prior to initiating ER hormone therapy.

"The body of evidence regarding endocrine therapy treatment outcomes for ER+ breast cancer patients based upon mTOR pathway activation status as measured by activation of specific mTOR kinase substrates is compelling," said Emanuel Petricoin, PhD, Co-Director of the Center for Applied Proteomics and Molecular Medicine (CAPMM) at George Mason University. "The data provides a strong rationale for physicians to seek-out mTOR pathway activation status as measured by protein phosphorylation of specific mTOR kinase substrates as part of the standard biomarker profiling paradigm in this patient population. The mTOR pathway activation assay licensed to Avant by George Mason University is patent protected and provides a significant barrier to entry so that Avant can
commercialize this technology with the appropriate strategic plan under newly-appointed President & CEO Dr. Philippe Goix's leadership.

Avant has developed an analytically and clinically validated mTOR-pathway activation-focused assay that measures the activation state of specific mTOR protein kinases such as p70S6 and 4EBP1 that indicate mTOR pathway activation and endocrine resistance in the ER+ BC patient population. The Company's mTOR pathway activation assay is protected by the issued US patent #8,628,931 that describes predicting a response to chemotherapy based upon the assay. The Company is evaluating strategic options for commercially launching the assay.

"This data provides further evidence that we, as a medical community, can push precision medicine to higher levels," said Brian Harvey, Former Director of the Division of Gastroenterology Products at the Center for Drug Evaluation Research at the FDA. "Testing that optimizes treatment for each individual patient is the ultimate clinical goal."

**mTOR prognostic data for Tamoxifen response in ER+ Breast Cancer from five publications**


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**The mTOR effectors 4EBP1 and S6K2 are frequently coexpressed, and associated with a poor prognosis and endocrine resistance in breast cancer: a retrospective study including patients from the randomized Stockholm tamoxifen trials**

Elin Karlsson1*, Gizeh Pérez-Tenorio1, Risul Amin1, Josefine Bostner1, Lambert Skoog2, Tommy Fornander3, Dennis C Sgroi4, Bo Nordenskjöld1, Anna-Lotta Hallbeck1 and Olle Stål1

**Abstract**

Introduction: mTOR and its downstream effectors the 4E-binding protein 1 (4EBP1) and the p70 ribosomal S6 kinases (S6K1 and S6K2) are frequently upregulated in breast cancer, and assumed to be driving forces in tumourigenesis, in close connection with estrogen receptor (ER) networks. Here, we investigated these factors as clinical markers in five different cohorts of breast cancer patients.

Methods: The prognostic significance of 4EBP1, S6K1 and S6K2 mRNA expression was assessed with real-time PCR in 93 tumours from the treatment randomized Stockholm trials, encompassing postmenopausal patients enrolled between 1976 and 1990. Three publicly available breast cancer cohorts were used to confirm the results. Furthermore, the predictive values of 4EBP1 and p4EBP1_S65 protein expression for both prognosis and endocrine treatment benefit were assessed by immunohistochemical analysis of 912 node-negative breast cancers from the Stockholm trials.

Results: S6K2 and 4EBP1 mRNA expression levels showed significant correlation and were associated with a poor outcome in all cohorts investigated. 4EBP1 protein was
confirmed as an independent prognostic factor, especially in progesterone receptor (PgR)-expressing cancers. 4EBP1 protein expression was also associated with a poor response to endocrine treatment in the ER/PgR positive group. Cross-talk to genomic as well as non-genomic ER/PgR signaling may be involved and the results further support a combination of ER and mTOR signaling targeted therapies.

Conclusion: This study suggests S6K2 and 4EBP1 as important factors for breast tumourigenesis, interplaying with hormone receptor signaling. We propose S6K2 and 4EBP1 as new potential clinical markers for prognosis and endocrine therapy response in breast cancer.


Phosphorylated p-70S6K predicts tamoxifen resistance in postmenopausal breast cancer patients randomized between adjuvant tamoxifen versus no systemic treatment

Karin Beelen1, Mark Opdam1, Tesa M Severson1, Rutger HT Koornstra1, Andrew D Vincent2, Jelle Wesseling3, Jettie J Muris3, Els MJJ Berens4, Jan B Vermorken5, Paul J van Diest6 and Sabine C Linn1,6,7*

Abstract

Introduction: Activation of the phosphatidylinositol-3-kinase (PI3K) and/or mitogen-activated protein kinase (MAPK) pathways results in anti-estrogen resistance in vitro, but a biomarker with clinical validity to predict intrinsic resistance has not been identified. In metastatic breast cancer patients with previous exposure to endocrine therapy, the addition of a mammalian target of rapamycin (mTOR) inhibitor has been shown to be beneficial. Whether or not patients on adjuvant endocrine treatment might benefit from these drugs is currently unclear. A biomarker that predicts intrinsic resistance could potentially be used as companion diagnostic in this setting. We tested the clinical validity of different downstream-activated proteins in the PI3K and/or MAPK pathways to predict intrinsic tamoxifen resistance in postmenopausal primary breast cancer patients.

Methods: We recollected primary tumor tissue from patients who participated in a randomized trial of adjuvant tamoxifen (1-3 years) versus observation. After constructing a tissue micro-array, cores from 563 estrogen receptor α positive were immunostained for p-AKT(Thr308), p-AKT(Ser473), p-mTOR, p-p70S6K and p-ERK1/2. Cox proportional hazard models for recurrence free interval were used to assess hazard ratios and interactions between these markers and tamoxifen treatment efficacy.

Results: Interactions were identified between tamoxifen and p-AKT(Thr308), p-mTOR, p-p70S6K and p-ERK1/2. Applying a conservative level of significance, p-p70S6K remained significantly associated with tamoxifen resistance. Patients with p-p70S6K negative tumors derived significant benefit from tamoxifen (HR 0.24, P<0.0001), while patients whose tumor did express p-p70S6K did not (HR=1.02, P =0.95), P for interaction 0.004. In systemically untreated breast cancer patients, p-p70S6K was associated with a decreased
risk for recurrence. Conclusions: Patients whose tumor expresses p-p70S6K, as a marker of downstream PI3K and/or MAPK pathway activation, have a favorable prognosis, but do not benefit from adjuvant tamoxifen. A potential benefit from inhibitors of the PI3K/Akt/mTOR pathway in these patients needs to be further explored.


Impact of dual mTORC1/2 mTOR kinase inhibitor AZD8055 on acquired endocrine resistance in breast cancer in vitro

Nicola J Jordan1,4, Carol M Dutkowski1, Denise Barrow1, Huw J Mottram1, Iain R Hutcheson2, Robert I Nicholson1, Sylvie M Guichard3 and Julia MW Gee1*

Abstract

Introduction: Upregulation of PI3K/Akt/mTOR signaling in endocrine-resistant breast cancer (BC) has identified mTOR as an attractive target alongside anti-hormones to control resistance. RAD001 (everolimus/Afinitor®), an allosteric mTOR inhibitor, is proving valuable in this setting; however, some patients are inherently refractory or relapse during treatment requiring alternative strategies. Here we evaluate the potential for novel dual mTORC1/2 mTOR kinase inhibitors, exemplified by AZD8055, by comparison with RAD001 in ER+ endocrine resistant BC cells.

Methods: In vitro models of tamoxifen (TamR) or estrogen deprivation resistance (MCF7-X) were treated with RAD001 or AZD8055 alone or combined with anti-hormone fulvestrant. Endpoints included growth, cell proliferation (Ki67), viability and migration, with PI3K/AKT/mTOR signaling impact monitored by Western blotting. Potential ER cross-talk was investigated by immunocytochemistry and RT-PCR.

Results: RAD001 was a poor growth inhibitor of MCF7-derived Tamer and MCF7-X cells (IC50 ≥1 μM), rapidly inhibiting mTORC1 but not mTORC2/AKT signaling. In contrast AZD8055, which rapidly inhibited both mTORC1 and mTORC2/AKT activity, was a highly effective (P<0.001) growth inhibitor of TamR (IC50 18nM) and MCF7-X (IC50 24 nM), and of a further T47D-derived tamoxifen resistant model T47D-tamR (IC50 19 nM). AZD8055 significantly (P<0.05) inhibited resistant cell proliferation, increased cell death and reduced migration. Furthermore, dual treatment of TamR or MCF7-X cells with AZD8055 plus fulvestrant provided superior control of resistant growth versus either agent alone (P<0.05). Co-treating with AZD8055 alongside tamoxifen (P<0.01) or estrogen deprivation (P<0.05) also effectively inhibited endocrine responsive MCF-7 cells. Although AZD8055 inhibited estrogen receptor (ER) ser167 phosphorylation in TamR and MCF7-X, it had no effect on ER ser118 activity or expression of several ER-regulated genes, suggesting the mTOR kinase inhibitor impact was largely ER-independent. The capacity of AZD8055 for ER-independent activity was further evidenced by growth inhibition (IC5018 and 20 nM) of two acquired fulvestrant resistant models lacking ER. Conclusions: This is the first report demonstrating dual mTORC1/2 mTOR kinase inhibitors have potential to control acquired endocrine resistant BC, even under conditions where everolimus fails. Such inhibitors may prove of particular benefit when used alongside anti-hormonal treatment as second-line
therapy in endocrine resistant disease, and also potentially alongside anti-hormones during the earlier endocrine responsive phase to hinder development of resistance.


Activation of Akt, mTOR, and the estrogen receptor as a signature to predict tamoxifen treatment benefit

Josefine Bostner, Elin Karlsson, Muneeswaran J. Pandiyan, Hanna Westman, Lambert Skoog, Tommy Fornander, Bo Nordenskjo, Olle Stal

Abstract

The frequent alterations of the PI3K/Akt/mTOR growth signaling pathway are proposed mechanisms for resistance to endocrine therapy in breast cancer, partly through regulation of estrogen receptor a (ER) activity. Reliable biomarkers for treatment prediction are required for improved individualized treatment. We performed a retrospective immunohistochemical analysis of primary tumors from 912 postmenopausal patients with node-negative breast cancer, randomized to either tamoxifen or no adjuvant treatment. Phosphorylated (p)Akt-serine(s)473,p-mTOR-s2448, and ER phosphorylations-s167 and -s305 were evaluated as potential biomarkers of prognosis and tamoxifen treatment efficacy. High expression of p-mTOR indicated a reduced response to tamoxifen, most pronounced in the ER+/progesterone receptor (PgR) + subgroup (tamoxifen vs. no tamoxifen: hazard ratio (HR), 0.86; 95 % confidence interval (CI), 0.31-2.38; P = 0.78), whereas low p-mTOR expression predicted tamoxifen benefit (HR, 0.29; 95 % CI, 0.18-0.49; P = 0.000002). In addition, nuclear p-Akt-s473 as well as p-ER at -s167 and/or -s305 showed interaction with tamoxifen efficacy with borderline statistical significance. A combination score of positive pathway markers including p-Akt, p-mTOR, and p-ER showed significant association with tamoxifen benefit (test for interaction; P = 0.029). Cross-talk between growth signaling pathways and ER-signaling has been proposed to affect tamoxifen response in hormone receptor-positive breast cancer. The results support this hypothesis, as an overactive pathway was significantly associated with reduced response to Tamoxifen. A clinical pretreatment test for cross-talk markers would be a step toward individualized adjuvant endocrine treatment with or without the addition of PI3K/Akt/mTOR pathway inhibitors.


PIK3CA mutations, phosphatase and tensin homolog, human epidermal growth factor receptor 2, and insulin-like growth factor 1 receptor and adjuvant tamoxifen resistance in postmenopausal breast cancer patients

Karin Beelen1, Mark Opdam1, Tesa M Severson1, Rutger HT Koornstra1, Andrew D Vincent2, Jelle Wesseling3, Jettie J Muris3, Els MJJ Berns4, Jan B Vermorken5, Paul J van Diest6 and Sabine C Linn1,6,7*
Abstract

Introduction: Inhibitors of the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway can overcome endocrine resistance in estrogen receptor (ER) α-positive breast cancer, but companion diagnostics indicating PI3K/AKT/mTOR activation and consequently endocrine resistance are lacking. PIK3CA mutations frequently occur in ERα-positive breast cancer and result in PI3K/AKT/mTOR activation in vitro. Nevertheless, the prognostic and treatment-predictive value of these mutations in ERα-positive breast cancer is contradictory. We tested the clinical validity of PIK3CA mutations and other canonic pathway drivers to predict intrinsic resistance to adjuvant tamoxifen. In addition, we tested the association between these drivers and downstream activated proteins.

Methods: Primary tumors from 563 ERα-positive postmenopausal patients, randomized between adjuvant tamoxifen (1 to 3 years) versus observation were recollected. PIK3CA hotspot mutations in exon 9 and exon 20 were assessed with Sequenom Mass Spectrometry. Immunohistochemistry was performed for human epidermal growth factor receptor 2 (HER2), phosphatase and tensin homolog (PTEN), and insulin-like growth factor 1 receptor (IGF-1R). We tested the association between these molecular alterations and downstream activated proteins (like phospho-protein kinase B (p-AKT), phospho-mammalian target of rapamycin (p-mTOR), p-ERK1/2, and p-p70S6K). Recurrence-free interval improvement with tamoxifen versus control was assessed according to the presence or absence of canonic pathway drivers, by using Cox proportional hazard models, including a test for interaction.

Results: PIK3CA mutations (both exon 9 and exon 20) were associated with low tumor grade. An enrichment of PIK3CA exon 20 mutations was observed in progesterone receptor- positive tumors. PIK3CA exon 20 mutations were not associated with downstream-activated proteins. No significant interaction between PIK3CA mutations or any of the other canonic pathway drivers and tamoxifen-treatment benefit was found.

Conclusion: PIK3CA mutations do not have clinical validity to predict intrinsic resistance to adjuvant tamoxifen and may therefore be unsuitable as companion diagnostic for PI3K/AKT/mTOR inhibitors in ERα- positive, postmenopausal, early breast cancer patients.

About Avant Diagnostics, Inc.

Avant is a healthcare information solutions company that specializes in biomarker tests that are being developed in the areas of oncology and neurology. Avant provides personalized medicine diagnostic testing capabilities through its TheraLink® diagnostic assays, initially for breast cancer, to assist clinical oncologists in identifying likely responders for over 30 FDA-approved drug treatment regimens. Avant is the leading developer of phospho-proteomic technologies for measuring the activation status of key signaling pathways, with applications across several different cancer types, including breast, ovarian, colorectal and pancreatic, that are instrumental in the development of companion diagnostics for molecular-targeted therapies. Avant has used these proteomic technologies to support the drug development programs of many of the top biopharmaceutical companies in the world. More information can be found at the website

Avant is also developing OvaDx® for use in monitoring women diagnosed previously with ovarian cancer. OvaDx® is a sophisticated proteomic microarray-based test that measures the activation of the immune system markers in blood samples in response to ovarian tumor cell development.

Avant's neurology division was recently acquired from Amarantus Bioscience Holdings, Inc. (OTC: AMBS) and owns certain rights to next-generation DNA sequencing (NGS) assay for the identification of patients with autoimmune disorders, and has an exclusive license to The LymPro Test™ for Alzheimer's disease, which was developed by Prof. Thomas Arendt, Ph.D., from the University of Leipzig. The Company also owns intellectual property for the proteomic-based diagnosis of Parkinson's disease (NuroPro), and other cell-cycle-related disorders.

For further information please visit http://www.Avantdiagnostics.com.

Forward-Looking Statements

Certain statements in this press release are forward-looking within the meaning of the Private Securities Litigation Reform Act of 1995. These statements may be identified by the use of forward-looking words such as "anticipate," "believe," "forecast," "estimated" and "intend," among others. These forward-looking statements are based on Avant's current expectations and actual results could differ materially. There are a number of factors that could cause actual events to differ materially from those indicated by such forward-looking statements. These factors include, but are not limited to, substantial competition; our ability to continue as a going concern; our need for additional financing; uncertainties with respect to lengthy and expensive clinical trials, that results of earlier studies and trials may not be predictive of future trial results; and risks related to failure to obtain FDA clearances or approvals and noncompliance with FDA regulations. There are no guarantees that future clinical trials discussed in this press release will be completed or successful, or that any product will receive regulatory approval for any indication or prove to be commercially successful. Avant does not undertake an obligation to update or revise any forward-looking statement except as required by law. Investors should read the risk factors set forth in Avant's Form 10-K filed with the Securities and Exchange Commission on January 13, 2016, and other periodic reports filed with the Securities and Exchange Commission.

Investor and Media Contact:
Ascendant Partners, LLC
Richard Galterio
+1-732-410-9810
rich@ascendantpartnersllc.com

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