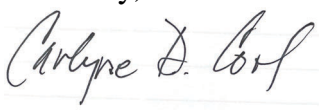
Dear John Doe,

Please find the Theralink Assay report for Jane Doe.
We greatly appreciate your support and look forward to working with you again.

Sincerely,



Carlyne D. Cool, M.D.
Medical Director
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Patient

Date of Birth: **01/01/1901**
Sex: **Female**
MRN: **1234567**
Theralink ID: **TT23-00089**

Specimen

Specimen ID: **AB00-12345-67**
Specimen Type: **Resection**
Collection Site: **Breast, left**

Timeline

Specimen Collected: **01/01/1901**
Test Ordered: **9/1/2023**
Specimen Received: **9/8/2023**

Provider

John Doe - Test Cancer Center
1234 Test Center Road, Suite 1111, Warrenton, VA 20187, Phone: 123-456-7890

Diagnosis and Treatment History

Diagnosis: **Invasive ductal carcinoma with extensive necrosis**
Stage: **Stage III**
Histology: **Invasive carcinoma of no special type (ductal)**

Line of Therapy: Declined Chemo

Hormonal Status

ER: **Negative** | PR: **Negative** | HER2: **Negative**

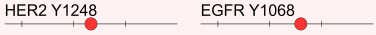
On-Label Options

anti-PD1 agent, such as pembrolizumab

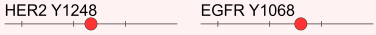
Off-Label Options

anti-HER2 agent, such as combination treatment: pertuzumab + trastuzumab + hyaluronidase-zzxf

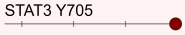
EGFR/HER2 kinase inhibitor, such as lapatinib, neratinib



HER2/HER3 kinase inhibitor, such as tucatinib



JAK/STAT3 inhibitor



SRC inhibitor

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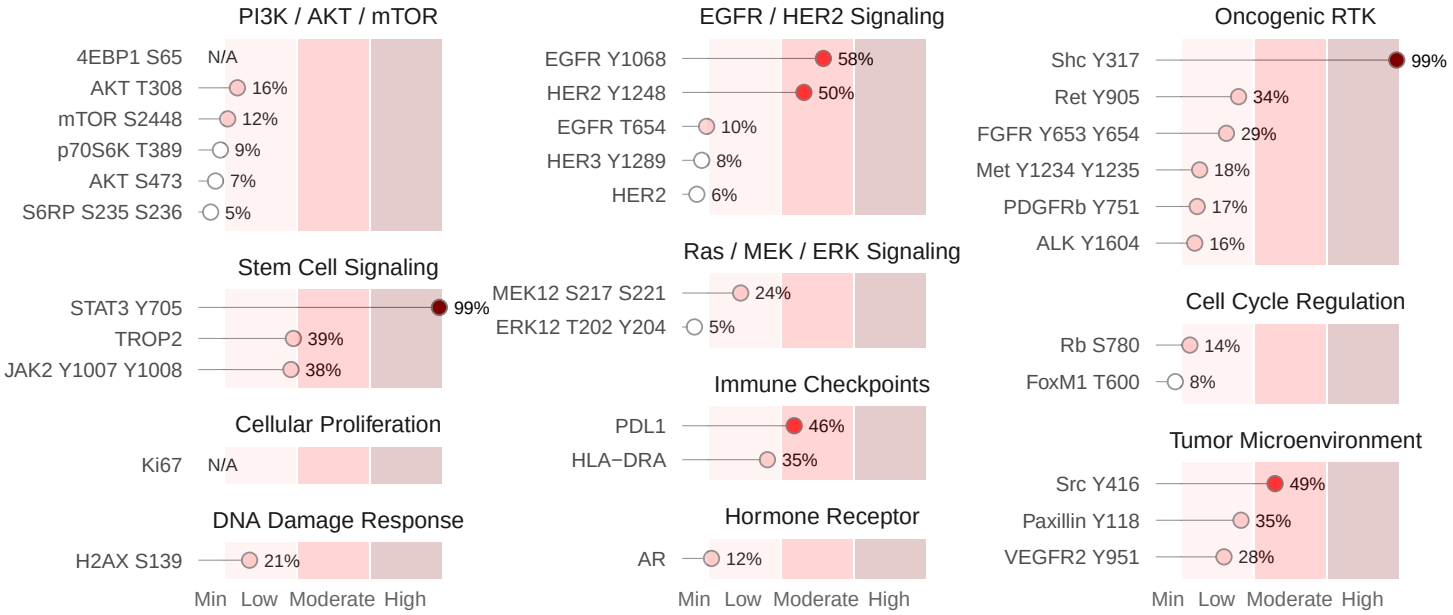
Complete Assay Results



The Theralink Assay utilizes a Reverse Phase Protein Array (RPPA) panel test to quantify the expression of proteins and phosphoproteins. Individual biomarkers are reported as a percentage, scaled to the Theralink reference population of primary breast tumor samples.

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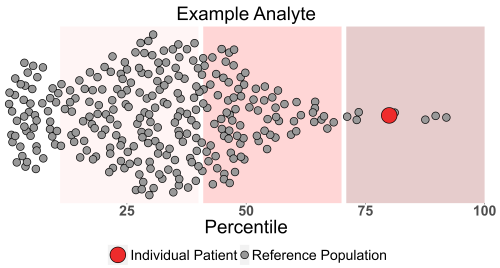
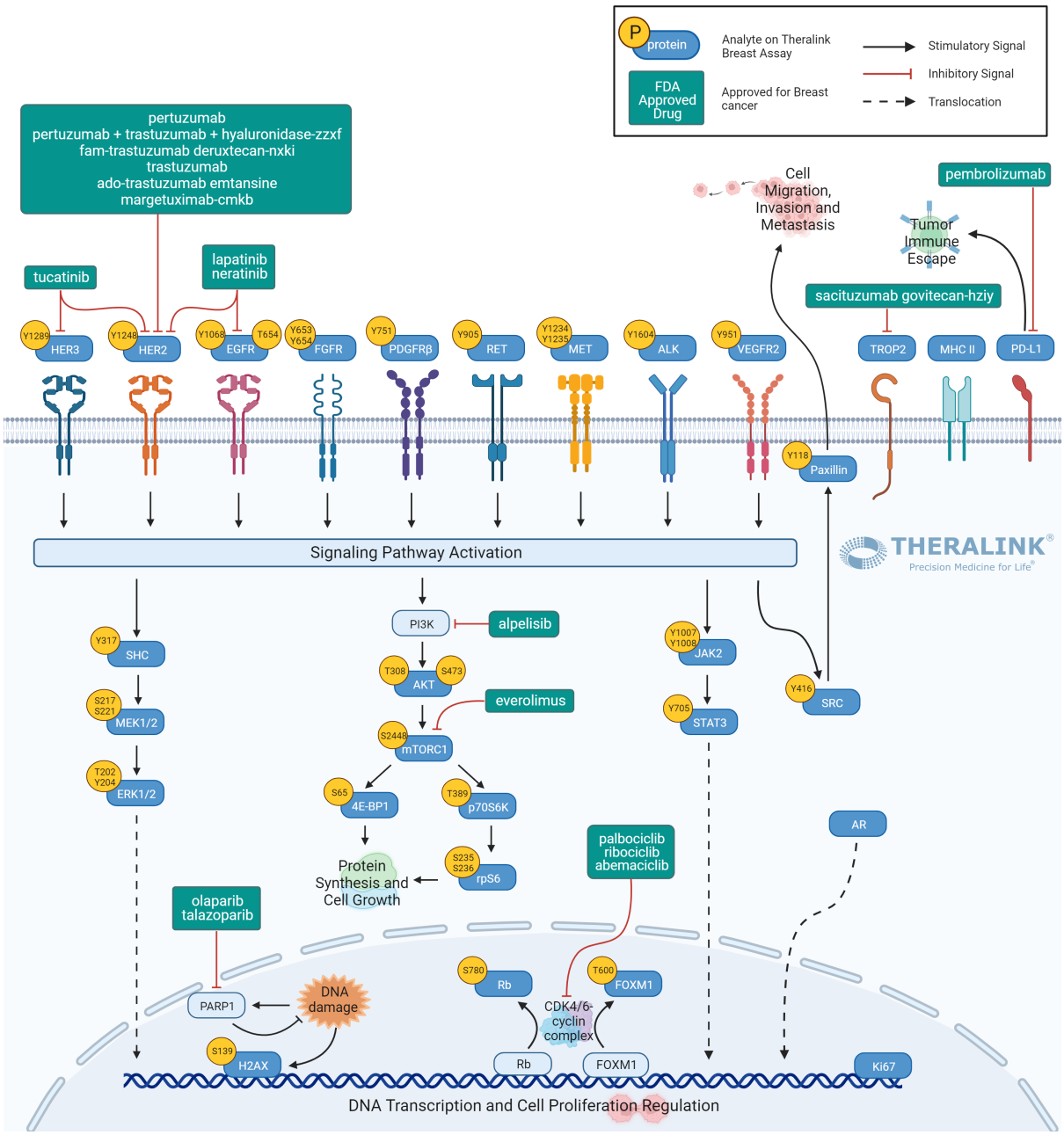
Method: The Theralink Assay utilizes a Reverse Phase Protein Array (RPPA) methodology to quantify the expression of proteins and phosphoproteins. Individual biomarkers are reported as a percentile, scaled to the Theralink reference population of primary breast tumor samples. Assay limits of sensitivity for total protein input are >= 100 ug/ml and <= 400 ug/ml. Limits of sensitivity for individual analytes don't apply due to reporting as percentile.





PI3K/AKT/mTOR Signaling

AKT is a serine/threonine-specific protein kinase group with three isoforms encoded by three different genes: AKT1, AKT2 and AKT3. This group of kinases represent a pivotal component in the PI3K/AKT/mTOR axis of signaling, regulating targets involved in multiple cellular processes such as metabolism, growth, survival, and proliferation (PMID: 15023437; 17604717). Full activation of AKT typically requires phosphorylation at T308 by PDK1, as well as at S473 by mTORC2 (PMID: 8978681; 9094314). Aberrant activity of AKT, via amplification or hyperactivation, has been described in several cancers, even in the presence of normal levels of upstream regulators PI3K and/or PTEN (PMID: 17604717; 16288292; 25309720). One of the most common causes of resistance against ERBB-targeted therapies is directly related to the compensatory overactivation of AKT, making it an important biomarker and promising target for drug development (PMID: 24463007). AKT-specific inhibitors (e.g., MK-2206) are currently in clinical trials (PMID: 33995085). Due to the complex integration of PI3K/AKT/mTOR signaling, and to avoid compensatory signaling, a multi-target inhibitory approach within PI3K, AKT, and mTOR signaling pathways is advisable.

Mammalian target of rapamycin (**mTOR**) is one of the main transducers in PI3K/AKT signaling and regulates metabolism, protein synthesis, cell growth and cell division (PMID: 28283069). mTOR acts as a catalytic subunit in two different protein complexes: mTORC1 (in association with Raptor) and mTORC2 (in association with Rictor). As a response to growth factor signaling and/or other upstream regulators, mTOR is activated via phosphorylation at S2448 (PMID: 19487463; 19145465). Abnormally activated mTOR promotes protein and lipid synthesis and maintains tumor proliferation (PMID: 19948145; 25688110). Like AKT, a multitarget inhibitory approach using mTOR inhibitors (e.g., everolimus, temsirolimus) and PI3K/AKT inhibitors may be beneficial to avoid compensatory signaling.

Eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) is a direct inhibitor of translation initiation factor 4E (**eIF4E**). It is a downstream component of mTOR signaling, disassociating from eIF4E once phosphorylated at S65 and/or T70 to allow the translation complex to assemble and protein synthesis to take place (PMID: 19339977). Phosphorylated 4EBP1 is a marker of mTOR activity and a potential prognosis indicator, correlating with tumor aggressiveness and poor outcome in both breast and endometrial cancers (PMID: 27026382; 19428047; 17200342).

Both, S6 ribosomal protein kinase (**p70S6K**) and S6 protein (**rpS6**) are downstream effectors of mTOR signaling and, like 4EBP1, play a crucial role in the regulation of protein translation (PMID: 15314020; 15659337). Phosphorylation of p70S6K at T389 is indicative of mTOR activation (PMID: 7489717; 9271440; 9465032) and leads to phosphorylation of rpS6 at multiple residues (e.g., S235, S236). Increased phosphorylation of p70S6K and rpS6 indicate potential sensitivity to PI3K/Akt/mTOR inhibitors (PMID: 22531277; 22213594; 22167413).

Cell Cycle Regulation

The transcription factor Forkhead Box M1 (**FoxM1**) is a key regulator of cell cycle progression, proliferation, differentiation, apoptosis, and angiogenesis, among other processes (PMID: 33680951). This oncogenic protein is a downstream target for several signaling cascades, including MAPK/ERK and PI3K/Akt pathways, and its activity is regulated by multiple posttranslational modifications. Phosphorylation of several residues, including T600, by cyclin-CDK complexes renders FoxM1 active during G2 phase (PMID: 18285455). Like Rb, FoxM1 activity may be indicative of sensitivity to CDK4/6-inhibitors (e.g., palbociclib, abemaciclib and ribociclib), which are standard of care treatment choices for hormone receptor positive breast cancers.

Retinoblastoma protein (**Rb**) is a tumor suppressor that controls cell division by regulating the G1/S transition of the cell cycle. Rb binds E2F transcription factors and acts as a co-repressor of genes necessary for DNA synthesis, thus preventing cell cycle progression. Once phosphorylated by cyclin-CDK complexes, Rb dissociates from E2F, allowing the latter to become active and propel the cell through the S phase (PMID: 10499802; 16936740). CDK4/6-inhibitors are standard of care treatment choices for hormone receptor positive breast cancers (e.g., palbociclib, abemaciclib and ribociclib). Phosphorylated Rb allows assessment of CDK4/6 activation and is therefore a potent indicator of tumor response/therapy resistance to CDK inhibitors (PMID: 24919854; 29050219; 22383795).

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EGFR/HER2 Signaling

The transmembrane receptor **HER2** belongs to the human epidermal growth factor receptor (HER/EGFR/ERBB) family and is expressed at low levels to help control cell growth, differentiation, and breast development/maturation. When HER2 forms a dimer—with either another HER2 protein or a different ERBB member—autophosphorylation of tyrosine residues within the intracellular domain triggers proliferative signaling cascades such as the MAPK/ERK and PI3K/Akt/mTOR pathways (PMID: 17471238; 21204711; 31455202). HER2/ERBB2 overexpression/amplification occurs in approximately 20-30

HER3 is encoded by the ERBB3 gene, and like HER2, requires dimerization for its activation. However, unlike other ERBB members, HER3 is kinase-impaired and can only be activated via transphosphorylation by a heterodimer binding partner, which is most commonly HER2 (PMID: 8816440; 24269963). Phosphorylation of tyrosine 1289 is a hallmark of HER3 activation and that of its downstream PI3K/Akt/mTOR signaling pathway (PMID: 7929151). HER3 activation has been associated with resistance to EGFR/HER2-targeted therapies as well as endocrine therapies in ER-positive patients. Anti-HER3 antibody treatment in combination with anti-ERBB and/or estrogen-antagonists are showing promising results in multiple studies (PMID: 30057690).

EGFR is one of the most important molecular targets within the ERBB family of receptors. Its overexpression is associated with large tumor size and poor prognosis (PMID: 2884496; PMID: 7612182) and is frequently observed in the more aggressive subtypes of breast cancer: triple-negative breast cancer (TNBC) as well as inflammatory breast cancer (IBC) (PMID: 20164687; 17146782; 2563719). EGFR activates signaling pathways involved in cell proliferation, motility, invasion and angiogenesis, such as MAPK/ERK, PI3K/Akt/mTOR, and PLC-γ1-PKC signaling (PMID: 22161825; 28513565). Phosphorylation of EGFR at Y1068 is a potent predictor of TKI response. Additionally, phosphorylation at T654 is a hallmark of enhanced receptor stability with a strong correlation with metastasis (PMID: 26247735; 31597954). Small molecule TKI specifically targeting EGFR1 have been FDA approved for breast cancer treatment (e.g., gefitinib, erlotinib) (PMID: 31756933), while monoclonal antibodies against EGFR1 that are currently approved for other cancer types (e.g., cetuximab, panitumumab) are being investigated for their use in breast cancer in clinical trials.

RAS/MEK/ERK Signaling

MEK1/2 activated via phosphorylation at S217/S221 by Raf protein kinases upon growth factor or cytokine stimulation. Once active, MEK1/2 phosphorylate extracellular signal-regulated kinases ERK1/2 at T202/Y204, which in turn activate a collection of downstream kinases and transcription factors that orchestrate cell cycle and survival (PMID: 16393692; 19935650; 17496918). MEK activation has been linked to drug resistance targeting oncogenic EGFR, Ras and Raf, while inhibitors of MEK (e.g., trametinib, selumetinib) alone or in combination with B-Raf inhibitors are showing promising results (PMID: 26399658; 28073102; 28954413; 29488071; 33402199). Similarly, ERK1/2 inhibitors (e.g., SCH772984, ASN007, ravoxertinib) are being considered for their antiproliferative properties even in conditions of resistance to B-Raf and MEK inhibitors (PMID: 34337566; 23614898; 27227380).

DNA Damage Response

Histone **H2AX** undergoes phosphorylation at S139 as part of the DNA repair response to double-strand breakage, which in turn is a major cause of malignant transformation. Elevated phosphorylated H2AX (γH2AX) is associated with failure of DNA repair and lack of genomic stability that drives tumorigenesis. In breast cancer, higher levels of γH2AX are associated with greater tumor size, higher grade and the spread to lymph nodes (PMID: 9488723; 26667849; 28158293; 31552812). At the same time, elevated γH2AX may indicate increased sensitivity to radiation, platinum-based chemotherapies and the combinations of the latter with DNA-repair inhibitors (e.g., FDA approved PARP1 inhibitor, Olaparib) (PMID: 18256616; 30429212; 19005492; 28158293).

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Oncogenic RTK Signaling

Fibroblast growth factor receptors (**FGFR**) are a family of receptor tyrosine kinases (RTKs) that promote cell proliferation, survival and migration. In cancer, FGFRs are frequently found aberrantly activated, sometimes due to amplification, gene fusion and hyperactivation (PMID: 8622701; 20094046; 32879300; 33268819). Several multi-target (e.g., pazopanib, ponatinib, regorafenib) and FGFR specific (e.g., erdafitinib, pemigatinib, and infigratinib) TKIs are available that should be considered depending on the signaling pathway activation context (i.e., elevated FGFR activation together with multiple other RTKs versus constitutively activated FGFR) (PMID: 31161538; 34720591; 35494629; doi:10.1038/s42004-021-00623-x).

Like other RTKs, the platelet-derived growth factor receptor beta (**PDGFRb**) gets phosphorylated (e.g., at Y751) upon ligand binding and dimerization, activating other signaling pathways, such as MAPK/ERK and PI3K/Akt. This receptor is known to regulate mesenchymal cells, like fibroblasts and pericytes, and is highly expressed in tumor stroma where it is found to stimulate tumor growth and angiogenesis (PMID: 1284870; 15207817; 18483217). High PDGFRb expression and dysregulated PDGF signaling activity strongly correlate with tumor aggressiveness and shorter survival (PMID: 19498003; 28970051; 29380207; 23583284). Several muti-target TKIs with anti-PDGFRb activity have been approved for cancer treatment (e.g., lenvatinib, regorafenib, pazopanib, sunitinib, ponatinib).

The receptor tyrosine kinase **RET** is activated by glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) and its subsequent phosphorylation in multiple tyrosine residues (including Y905) results in the activation of downstream signaling pathways, such as MAPK/ERK (PMID: 15982921; 32993133). Oncogenic mutations that result in overexpression and/or constitutive activation of RET are commonly found in several tumor types and are associated with tumor growth, invasion, and metastasis, especially in pancreatic, prostate, and breast cancers (PMID: 30666215). In the context of activated RET, therapy options include multiple small molecule multi-kinase TKIs (e.g., sunitinib, ponatinib, vandetanib, lenvatinib, and sorafenib) and RET-selective inhibitors (pralsetinib and selpercatinib) (PMID: 28911727; 29134959; 3066621; 34497761).

Like other RTKs, **MET** (mesenchymal-epithelial transition factor) plays an essential role in the regulation of cell proliferation, survival and migration (PMID: 21102609). Activation of MET via the ligand hepatocyte growth factor (HGF) and homodimerization results in tyrosine autophosphorylation (i.e., Y1234/Y1235) and the activation of canonical downstream signaling cascades, such as MAPK/ERK, PI3K/Akt and STAT3 pathways (PMID: 11114738; 21102609; 22128289). Aberrant activation of MET — due to mutations and/or crosstalk with ERBB members (e.g., EGFR) — has been found to play a major role in multiple malignancies, including breast, ovarian, liver, lung and pancreatic carcinomas (PMID: 18709663; 22270953). Such dysregulated MET activity has been associated with cancer progression, invasiveness, and therapy resistance, especially in EGFR-dependent cancers (PMID: 17463250: 27450722; 32435640). For instance, EGFR-TKI-resistant/MET-amplified non-small cell lung cancer (NSCLC) patients are showing better results when targeting EGFR in combination with MET (using crizotinib, tivantinib, tepotinib, and cabozantinib, onartuzumab, etc.) or HGF (using ficlatuzumab, rilotumumab, etc.) (PMID: 29621416; 29121501; 31227004; 34322251).

Anaplastic lymphoma kinase (**ALK**) is a RTK that is upstream of several proliferative signaling pathways, such as MAPK/ERK, PI3K/Akt/mTOR, and phospholipase C gamma, among others. Oncogenic ALK signaling is typically found due to genomic rearrangements. The ALK locus is a hot spot for translocation that often results in chimeric fusions with other genes that provide ALK with higher expression rates and constitutive activation (e.g., NPM1-ALK in ALCL and EML4-ALK in NSCLC) (PMID: 24060861; 26384210; 28122866). Phosphorylation of ALK at Y1604 is necessary for its activation and a biomarker of ALK oncogenic signaling as well as a readout for sensitivity to ALK-inhibitors such as alectinib and crizotinib (PMID: 9819383; 17483355; 21847362; 34423228; 34994610).

Shc is an adaptor protein that relays RTK signaling to downstream MAPK/ERK and PI3K/Akt pathways. Shc phosphorylation at Y239, Y240 and Y317 results in recruitment of Grb2/SOS complexes that lead to Ras activation (PMID: 1465135; 18279888; 30210578). Shc is a major mediator of HER2/ERBB signaling, and increased phosphorylation in breast tumors has been associated with poor prognosis, including metastasis and relapse despite tamoxifen therapy (PMID: 9696394; 10741744; 17196107; 18604176; 24407288).

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Cellular Proliferation

Antigen **Ki-67** is a nuclear non-histone protein and a canonical proliferation marker. It is highly expressed in actively dividing cells (PMID: 6339421; 1484317) and indicative of responsiveness to chemotherapy (PMID: 19436038; 22993598; 22993598; 23674192; 25049332).

Androgen Signaling

Androgen receptor (**AR**) signaling has been well known for its role in prostate cancer where it has been a critical therapy target. Elevated AR levels have also been seen in other types of cancer, including breast cancer, where it has shown similar tumorigenic properties as well as potential as a therapeutic target, especially in AR-positive, ER-negative/PR-negative breast cancers and TNBC (PMID: 4124279; 23965901; 27816190; 28085048). AR inhibitors have been approved to treat prostate cancer (e.g., bicalutamide and abiraterone acetate) and are currently being tested for their use in breast cancer patients in multiple trials (PMID: 31952272).

Tumor Microenvironment

VEGFR2 is one of the main RTKs responsible for the transduction of angiogenic and vascular permeability signaling. After binding of its cognate ligand VEGF-A and subsequent tyrosine autophosphorylation (e.g., Y951), VEGFR2 activates multiple downstream effectors to promote endothelial proliferation and migration. Some of these effectors include: the PLCgamma/MAPK/ ERK proliferative pathway; Src kinases, which regulate cell-to-cell contacts as well as survival via PI3K/Akt; and the SHB/FAK/paxillin axis, which is necessary for focal adhesion turnover and motility (PMID: 16006559; 22866201). VEGF-A/VEGFR2 signaling is one of the main drivers of tumor vascularization, growth and progression, and a major target for anti-angiogenesis therapies (PMID: 12778165; 18463380). Such therapies consist of small molecule inhibitors (e.g., sorafenib, axitinib and pazopanib) as well as monoclonal antibodies targeting VEGF (e.g., bevacizumab) or VEGFR2 (e.g., ramucirumab) (PMID: 26500608; 29508855; 35281942).

Src is a non-receptor tyrosine kinase known for its role in several signaling pathways and a key player in the regulation of cell division, survival, angiogenesis, and adhesion. When phosphorylated (e.g., Y416), this kinase regulates the activity of cadherins, catenins, FAK/Paxillin and Akt, among other effectors (PMID: 18487549; 22153719; 34193161). Increased Src activity strongly correlates with—and most likely contributes to—higher tumor malignancy, invasion, metastasis and TKI resistance (PMID: 6403227; 9014858; 12884910; 15170449; 18487549; 19581523; 21399647). While Src family TKIs are available (i.e. dasatinib), possible associations with immunosuppression warrant careful consideration (PMID: 25662515; 30404626; 34193161).

Paxillin is a downstream signaling adaptor and focal adhesion scaffolding protein with an important role in normal and pathological cell migration. Upon integrin binding and phosphorylation by Src/FAK (e.g., Y118), Paxillin recruits other cotransducer proteins to regulate the adhesion complex dynamics (i.e., assembly versus turnover) and the cytoskeletal rearrangements that are necessary for cell motility (PMID: 28214467; 32859368). Increased Paxillin phosphorylation has been associated with tumor cell growth, migration, invasion and metastasis, and indicates possible responsiveness to Src/FAK inhibitors (PMID: 16360410; 17319853; 23226574).

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Stem Cell Signaling

The **JAK2/STAT3** pathway plays a major role mediating cytokine signaling (e.g., IL-6, TNF-gamma) and is found active in both normally developing tissues as well as in multiple cancer types (PMID: 26151455; 31952344; 35241923). JAK2 is a non-receptor tyrosine kinase which is responsible for the phosphorylation of transcription factor STAT3 (i.e., Y705) (PMID: 9111318; 28714740). Activated STAT3 homodimers translocate to the nucleus and promote the transcription of genes that are necessary for cell proliferation, inflammation, survival, metastasis, and epithelial–mesenchymal transition (EMT) (PMID: 17216035; 31952344; 21633165; 27003603; 31308780; 31952344; 32111215). In addition, JAK2/STAT3 activation has been implicated in chemoresistance (e.g., tamoxifen and palbociclib resistance in ER+ breast cancer, resistance to PD-1 blockage).

Trophoblast cell-surface antigen-2 (**TROP2**) is a transmembrane glycoprotein and calcium signal transducer that is commonly found overexpressed in a variety of solid tumor types (including breast), in most cases correlating with poor prognosis and low overall and disease-free survival (PMID: 18813308; 24086649; 26716416; 27645103). TROP2 has become an attractive target for cancer therapies and important marker of treatment sensitivity, and it is particularly promising in triple negative breast cancer (TNBC), where traditional targets are absent. Sacituzumab govitecan is a TROP2-directed antibody and topoisomerase inhibitor drug conjugate that has been approved for use in TNBC patients (PMID: 25915780; 30881031; 33196706; 34116144).

Immune Checkpoints

Programmed death-ligand 1 (**PDL1**) is an immune checkpoint molecule which inhibits T cell activation upon binding to PD1. Under normal conditions, the PDL1/PD1 pathway plays an important role in keeping homeostasis and a proper amount of immune response to avoid pathogenic autoimmunity (PMID: 20636820; 25749122). While PDL1 is normally expressed at low levels in B and T cells, macrophages, and dendritic cells, it can also be found in tumor cells with expression levels that correlate with T cell suppression and tumor immune escape (PMID: 22437870). Breast cancer patients with higher PDL1 expression show more aggressive phenotypes and shorter survival times (PMID: 31359214). The PDL1/PD1 axis has been an important target for the development and use of checkpoint inhibitor immunotherapies, as well as a powerful biomarker to determine which patients will benefit the most from such treatments (PMID: 31336685; 31883913).

HLA-DR is a major histocompatibility complex (MHC) class II antigen presentation molecule, which, together with other MHC-II molecules (HLA-DP and HLA-DQ), presents antigenic peptides obtained from the extracellular environment. The expression of this complex is typically restricted to antigen presenting cells such as dendritic cells, B cells, and macrophages, where it is required for T cell activation and the regulation of adaptive immune responses. However, IFN-gamma stimulation can cause other cell types, such as tumor cells, to produce MHC-II molecules. In tumors, HLA-DR is sometimes highly expressed and associated with higher lymphocytic infiltration as well as better clinical outcomes and indicative of responsiveness to immune checkpoint inhibition (i.e., anti-PDL1/PD) and other immunotherapies (PMID: 2112515; 21281807; 2227249; 3283252; 9301532; 11156321; 30463850).

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Approved Breast Cancer Therapeutics

Abemaciclib is an inhibitor of cyclin-dependent kinases 4 and 6 (CDK4 and CDK6). These kinases are activated upon bindingto D-cyclins. In estrogen receptor-positive (ER+) breast cancer cell lines, cyclin D1 and CDK4/6 promote phosphorylation of the retinoblastoma protein (Rb), cell cycle progression, and cell proliferation. In vitro, continuous exposure to abemaciclib inhibited Rb phosphorylation and blocked progression from G1 into S phase of the cell cycle, resulting in senescence and apoptosis. In breast cancer xenograft models, abemaciclib dosed daily without interruption as a single agent or in combination with antiestrogens resulted in reduction of tumor size.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/208716s006s007s008lbl.pdf

Ado-trastuzumab emtansine is a HER2-targeted antibody-drug conjugate. The antibody is the humanized anti-HER2 IgG1, trastuzumab. The small molecule cytotoxin, DM1, is a microtubule inhibitor. Upon binding to sub-domain IV of the HER2 receptor, ado-trastuzumab emtansine undergoes receptor-mediated internalization and subsequent lysosomal degradation, resulting in intracellular release of DM1-containing cytotoxic catabolites. Binding of DM1 to tubulin disrupts microtubule networks in the cell, which results in cell cycle arrest and apoptotic cell death. In addition, in vitro studies have shown that similar to trastuzumab, ado-trastuzumab emtansine inhibits HER2 receptor signaling, mediates antibody-dependent cellmediated cytotoxicity and inhibits shedding of the HER2 extracellular domain in human breast cancer cells that overexpress HER2.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125427lbl.pdf

Alpelisib is an inhibitor of phosphatidylinositol-3-kinase (PI3K) with inhibitory activity predominantly against PI3Kα. Gain-offunction mutations in the gene encoding the catalytic α-subunit of PI3K (PIK3CA) lead to activation of PI3Kα and Akt-signaling, cellular transformation and the generation of tumors in in vitro and in vivo models. In breast cancer cell lines, alpelisib inhibited the phosphorylation of PI3K downstream targets, including Akt and showed activity in cell lines harboring a PIK3CA mutation. In vivo, alpelisib inhibited the PI3K/Akt signaling pathway and reduced tumor growth in xenograft models, including models of breast cancer. PI3K inhibition by alpelisib treatment has been shown to induce an increase in estrogen receptor (ER) transcription in breast cancer cells. The combination of alpelisib and fulvestrant demonstrated increased antitumor activity compared to either treatment alone in xenograft models derived from ER-positive, PIK3CA mutated breast cancer cell lines.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/212526s000lbl.pdf

Many breast cancers have estrogen receptors and growth of these tumors can be stimulated by estrogen. In postmenopausal women, the principal source of circulating estrogen (primarily estradiol) is conversion of adrenally-generated androstenedione to estrone by aromatase in peripheral tissues, such as adipose tissue, with further conversion of estrone to estradiol. Many breast cancers also contain aromatase; the importance of tumor-generated estrogens is uncertain. Treatment of breast cancer has included efforts to decrease estrogen levels, by ovariectomy premenopausally and by use of anti-estrogens and progestational agents both pre- and postmenopausally; and these interventions lead to decreased tumor mass or delayed progression of tumor growth in some women. **Anastrozole** is a potent and selective non-steroidal aromatase inhibitor. It significantly lowers serum estradiol concentrations and has no detectable effect on formation of adrenal corticosteroids or aldosterone.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020541s026lbl.pdf

Everolimus is an inhibitor of mammalian target of rapamycin (mTOR), a serine-threonine kinase, downstream of the PI3K/AKT pathway. The mTOR pathway is dysregulated in several human cancers. Everolimus binds to an intracellular protein, FKBP-12, resulting in an inhibitory complex formation with mTOR complex 1 (mTORC1) and thus inhibition of mTOR kinase activity. Everolimus reduced the activity of S6 ribosomal protein kinase (S6K1) and eukaryotic elongation factor 4E-binding protein (4EBP1), downstream effectors of mTOR, involved in protein synthesis. S6K1 is a substrate of mTORC1 and phosphorylates the activation domain 1 of the estrogen receptor which results in ligand-independent activation of the receptor. In addition, everolimus inhibited the expression of hypoxia-inducible factor (e.g., HIF-1) and reduced the expression of vascular endothelial growth factor (VEGF). Inhibition of mTOR by everolimus has been shown to reduce cell proliferation, angiogenesis, and glucose uptake in in vitro and/or in vivo studies. Constitutive activation of the PI3K/Akt/mTOR pathway can contribute to endocrine resistance in breast cancer. In vitro studies show that estrogen-dependent and HER2+ breast cancer cells are

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Approved Breast Cancer Therapeutics (continued)

sensitive to the inhibitory effects of everolimus, and that combination treatment with everolimus and Akt, HER2, or aromatase inhibitors enhances the anti-tumor activity of everolimus in a synergistic manner. Two regulators of mTORC1 signaling are the oncogene suppressors tuberin-sclerosis complexes 1 and 2 (TSC1, TSC2). Loss or inactivation of either TSC1 or TSC2 leads to activation of downstream signaling. In TSC, a genetic disorder, inactivating mutations in either the TSC1 or the TSC2 gene lead to hamartoma formation throughout the body.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/022334s016lbl.pdf

Breast cancer cell growth may be estrogen-dependent. Aromatase is the principal enzyme that converts androgens to estrogens both in pre-and postmenopausal women. While the main source of estrogen (primarily estradiol) is the ovary in premenopausal women, the principal source of circulating estrogens in postmenopausal women is from conversion of adrenal and ovarian androgens (androstenedione and testosterone) to estrogens (estrone and estradiol) by the aromatase enzyme in peripheral tissues. **Exemestane** is an irreversible, steroidal aromatase inactivator, structurally related to the natural substrate androstenedione. It acts as a false substrate for the aromatase enzyme, and is processed to an intermediate that binds irreversibly to the active site of the enzyme, causing its inactivation, an effect also known as "suicide inhibition." Exemestane significantly lowers circulating estrogen concentrations in postmenopausal women, but has no detectable effect on adrenal biosynthesis of corticosteroids or aldosterone. Exemestane has no effect on other enzymes involved in the steroidogenic pathway up to a concentration at least 600 times higher than that inhibiting the aromatase enzyme.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/208716s006s007s008lbl.pdf

Fam-trastuzumab deruxtecan-nxki is a HER2-directed antibody-drug conjugate. The antibody is a humanized anti-HER2 IgG1. The small molecule, DXd, is a topoisomerase I inhibitor attached to the antibody by a cleavable linker. Following binding to HER2 on tumor cells, fam-trastuzumab deruxtecan-nxki undergoes internalization and intracellular linker cleavage by lysosomal enzymes. Upon release, the membrane-permeable DXd causes DNA damage and apoptotic cell death.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/761139s011lbl.pdf

Many breast cancers have estrogen receptors (ER) and the growth of these tumors can be stimulated by estrogen. **Fulvestrant** is an estrogen receptor antagonist that binds to the estrogen receptor in a competitive manner with affinity comparable to that of estradiol and downregulates the ER protein in human breast cancer cells. In vitro studies demonstrated that fulvestrant is a reversible inhibitor of the growth of tamoxifen-resistant, as well as estrogen-sensitive human breast cancer (MCF-7) cell lines. In in vivo tumor studies, fulvestrant delayed the establishment of tumors from xenografts of human breast cancer MCF-7 cells in nude mice. Fulvestrant inhibited the growth of established MCF-7 xenografts and of tamoxifen-resistant breast tumor xenografts. Fulvestrant showed no agonist-type effects in in vivo uterotropic assays in immature or ovariectomized mice and rats. In in vivo studies in immature rats and ovariectomized monkeys, fulvestrant blocked the uterotrophic action of estradiol. In postmenopausal women, the absence of changes in plasma concentrations of FSH and LH in response to fulvestrant treatment (250 mg monthly) suggests no peripheral steroidal effects.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021344s034lbl.pdf

Lapatinib is a 4-anilinoquinazoline kinase inhibitor of the intracellular tyrosine kinase domains of both Epidermal Growth Factor Receptor (EGFR [ErbB1]) and of Human Epidermal Receptor Type 2 (HER2 [ErbB2]) receptors (estimated Ki app values of 3nM and 13nM, respectively) with a dissociation half-life of ≥300 minutes. Lapatinib inhibits ErbB-driven tumor cell growth in vitro and in various animal models. An additive effect was demonstrated in an in vitro study when lapatinib and 5-FU (the active metabolite of capecitabine) were used in combination in the 4 tumor cell lines tested. The rowth inhibitory effects of lapatinib were evaluated in trastuzumab-conditioned cell lines. Lapatinib retained significant activity against breast cancer cell lines selected for long-term growth in trastuzumab-containing medium in vitro. These in vitro findings suggest non-cross resistance between these two agents. Hormone receptor positive breast cancer cells (with ER [Estrogen Receptor] and/or PgR [Progesterone Receptor]) that coexpress the HER2 tend to be resistant to established endocrine therapies. Similarly, hormone receptor positive breast cancer cells that initially lack EGFR or HER2 upregulate these receptor proteins as the tumor becomes resistant to endocrine therapy.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022059s007lbl.pdf

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The growth of some cancers of the breast is stimulated or maintained by estrogens. Treatment of breast cancer thought to be hormonally responsive (i.e., estrogen and/or progesterone receptor positive or receptor unknown) has included a variety of efforts to decrease estrogen levels (ovariectomy, adrenalectomy, hypophysectomy) or inhibit estrogen effects (antiestrogens and progestational agents). These interventions lead to decreased tumor mass or delayed progression of tumor growth in some women. In postmenopausal women, estrogens are mainly derived from the action of the aromatase enzyme, which converts adrenal androgens (primarily androstenedione and testosterone) to estrone and estradiol. The suppression of estrogen biosynthesis in peripheral tissues and in the cancer tissue itself can therefore be achieved by specifically inhibiting the aromatase enzyme. **Letrozole** is a nonsteroidal competitive inhibitor of the aromatase enzyme system; it inhibits the conversion of androgens to estrogens. In adult nontumor-and tumor-bearing female animals, letrozole is as effective as ovariectomy in reducing uterine weight, elevating serum LH, and causing the regression of estrogen-dependent tumors. In contrast to ovariectomy, treatment with letrozole does not lead to an increase in serum FSH. Letrozole selectively inhibits gonadal steroidogenesis but has no significant effect on adrenal mineralocorticoid or glucocorticoid synthesis. Letrozole inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome P450 subunit of the enzyme, resulting in a reduction of estrogen biosynthesis in all tissues. Treatment of women with letrozole significantly lowers serum estrone, estradiol and estrone sulfate and has not been shown to significantly affect adrenal corticosteroid synthesis, aldosterone synthesis, or synthesis of thyroid hormones.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020726s027lbl.pdf

Margetuximab-cmkb binds to the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2). Upon binding to HER2-expressing tumor cells, margetuximab-cmkb inhibits tumor cell proliferation, reduces shedding of the HER2 extracellular domain and mediates antibody-dependent cellular cytotoxicity (ADCC). In vitro, the modified Fc region of margetuximab-cmkb increases binding to activating Fc receptor FCGR3A (CD16A) and decreases binding to inhibitory Fc receptor FCGR2B (CD32B). These changes lead to greater in vitro ADCC and NK cell activation.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/761150s000lbl.pdf

Neratinib is a kinase inhibitor that irreversibly binds to Epidermal Growth Factor Receptor (EGFR), Human Epidermal Growth Factor Receptor 2 (HER2), and HER4. In vitro, neratinib reduces EGFR and HER2 autophosphorylation, downstream MAPK and AKT signaling pathways, and showed antitumor activity in EGFR and/or HER2 expressing carcinoma cell lines. Neratinib human metabolites M3, M6, M7 and M11 inhibited the activity of EGFR, HER2 and HER4 in vitro. In vivo, oral administration of neratinib inhibited tumor growth in mouse xenograft models with tumor cell lines expressing HER2 and EGFR.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/208051s000lbl.pdf

Olaparib is an inhibitor of poly (ADP-ribose) polymerase (PARP) enzymes, including PARP1, PARP2, and PARP3. PARP enzymes are involved in normal cellular functions, such as DNA transcription and DNA repair. Olaparib has been shown to inhibit growth of select tumor cell lines in vitro and decrease tumor growth in mouse xenograft models of human cancer, both as monotherapy or following platinum based chemotherapy. Increased cytotoxicity and anti-tumor activity following treatment with olaparib were noted in cell lines and mouse tumor models with deficiencies in BRCA and non-BRCA proteins involved in the homologous recombination repair (HRR) of DNA damage and correlated with platinum response. In vitro studies have shown that olaparib-induced cytotoxicity may involve inhibition of PARP enzymatic activity and increased formation of PARPDNA complexes, resulting in DNA damage and cancer cell death.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208558s001lbl.pdf

Palbociclib is an inhibitor of cyclin-dependent kinases (CDK) 4 and 6. Cyclin D1 and CDK4/6 are downstream of signaling pathways which lead to cellular proliferation. In vitro, palbociclib reduced cellular proliferation of estrogen receptor (ER)-positive breast cancer cell lines by blocking progression of the cell from G1 into S phase of the cell cycle. Treatment of breast cancer cell lines with the combination of palbociclib and antiestrogens leads to decreased retinoblastoma (Rb) protein phosphorylation resulting in reduced E2F expression and signaling, and increased growth arrest compared to treatment with each drug alone. In vitro treatment of ER-positive breast cancer cell lines with the combination of palbociclib and

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antiestrogens led to increased cell senescence compared to each drug alone, which was sustained for up to 6 days following palbociclib removal and was greater if antiestrogen treatment was continued. In vivo studies using a patient-derived ER-positive breast cancer xenograft model demonstrated that the combination of palbociclib and letrozole increased the inhibition of Rb phosphorylation, downstream signaling, and tumor growth compared to each drug alone. Human bone marrow mononuclear cells treated with palbociclib in the presence or absence of an anti-estrogen in vitro did not become senescent and resumed proliferation following palbociclib withdrawal.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/207103s008lbl.pdf

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. **Pembrolizumab** is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/125514s096lbl.pdf

Pertuzumab targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2) and, thereby, blocks ligand-dependent heterodimerization of HER2 with other HER family members, including EGFR, HER3 and HER4. As a result, pertuzumab inhibits ligand-initiated intracellular signaling through two major signal pathways, mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase (PI3K). Inhibition of these signaling pathways can result in cell growth arrest and apoptosis, espectively. In addition, pertuzumab mediates antibody-dependent cell-mediated cytotoxicity (ADCC). While pertuzumab alone inhibited the proliferation of human tumor cells, the combination of pertuzumab and trastuzumab significantly augmented anti-tumor activity in HER2-overexpressing xenograft models.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125409lbl.pdf

Pertuzumab, trastuzumab, and hyaluronidase-zzxf - for mechanisms of action, see individual sections for pertuzumab and trastuzumab.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/761170s000lbl.pdf

Ribociclib is an inhibitor of cyclin-dependent kinase (CDK) 4 and 6. These kinases are activated upon binding to Dcyclins and play a crucial role in signaling pathways which lead to cell cycle progression and cellular proliferation. The cyclin D-CDK4/6 complex regulates cell cycle progression through phosphorylation of the retinoblastoma protein (pRb). In vitro, ribociclib decreased pRb phosphorylation leading to arrest in the G1 phase of the cell cycle and reduced cell proliferation in breast cancer cell lines. In vivo, treatment with single agent ribociclib in a rat xenograft model with human tumor cells led to decreased tumor volumes, which correlated with inhibition of pRb phosphorylation. In studies using patient-derived estrogen receptor positive breast cancer xenograft models, combination of ribociclib and antiestrogen (e.g. letrozole) resulted in increased tumor growth inhibition compared to each drug alone.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209092s000lbl.pdf

Sacituzumab govitecan-hziy is a Trop-2-directed antibody-drug conjugate. Sacituzumab is a humanized antibody that recognizes Trop-2. The small molecule, SN-38, is a topoisomerase I inhibitor, which is covalently attached to the antibody by a linker. Pharmacology data suggest that sacituzumab govitecan-hziy binds to Trop-2-expressing cancer cells and is internalized with the subsequent release of SN-38 via hydrolysis of the linker. SN-38 interacts with topoisomerase I and prevents re-ligation of topoisomerase I-induced single strand breaks. The resulting DNA damage leads to apoptosis and cell death. Sacituzumab govitecan-hziy decreased tumor growth in mouse xenograft models of triple-negative breast cancer.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/761115s009lbl.pdf

Talazoparib is an inhibitor of poly (ADP-ribose) polymerase (PARP) enzymes, including PARP1 and PARP2, which play a role in DNA repair. In vitro studies with cancer cell lines that harbored defects in DNA repair genes, including BRCA 1 and 2,

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have shown that talazoparib-induced cytotoxicity may involve inhibition of PARP enzymatic activity and increased formation of PARPDNA complexes resulting in DNA damage, decreased cell proliferation, and apoptosis. Talazoparib anti-tumor activity was observed in human patient-derived xenograft breast cancer tumor models that expressed mutated or wild-type BRCA 1 and 2.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/211651s000lbl.pdf

Tamoxifen is an estrogen agonist/antagonist. Tamoxifen competes with estrogen for binding to the estrogen receptor, which can result in a decrease in estrogen receptor signaling-dependent growth in breast tissue. Tamoxifen has demonstrated antitumor activity against human breast cancer cell lines xenografted in mice. The drug has been shown to inhibit the induction of rat mammary carcinoma induced by dimethylbenzanthracene (DMBA) and to cause the regression of already established DMBA-induced tumors.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/021807s005lbl.pdf

Toremifene is a nonsteroidal triphenylethylene derivative. Toremifene binds to estrogen receptors and may exert estrogenic, antiestrogenic, or both activities, depending upon the duration of treatment, animal species, gender, target organ, or endpoint selected. In general, however, nonsteroidal triphenylethylene derivatives are predominantly antiestrogenic in rats and humans and estrogenic in mice. In rats, toremifene causes regression of established dimethylbenzanthracene (DMBA)-induced mammary tumors. The antitumor effect of toremifene in breast cancer is believed to be mainly due to its antiestrogenic effects, i.e., its ability to compete with estrogen for binding sites in the cancer, blocking the growth-stimulating effects of estrogen in the tumor.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020497s006lbl.pdf

The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of 185 kDa, which is structurally related to the epidermal growth factor receptor. **Trastuzumab** has been shown, in both in vitro assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2. Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity (ADCC). In vitro, Trastuzumab-mediated ADCC has been shown to be preferentially exerted on HER2 overexpressing cancer cells compared with cancer cells that do not overexpress HER2.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/103792s5250lbl.pdf

Tucatinib is a tyrosine kinase inhibitor of HER2. In vitro, tucatinib inhibits phosphorylation of HER2 and HER3, resulting in inhibition of downstream MAPK and AKT signaling and cell proliferation, and showed antitumor activity in HER2 expressing tumor cells. In vivo, tucatinib inhibited the growth of HER2 expressing tumors. The combination of tucatinib and trastuzumab showed increased anti-tumor activity in vitro and in vivo compared to either drug alone.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/213411s000lbl.pdf

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