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International Congress on
TargetedTherapies
in Cancer

What Turns a Good Cell Bad?
Why New Theories on the "Hallmarks of Cancer" Matter to Community Oncologists

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Insight into oncology research.

In estrogen receptor-positive
(ER+) advanced breast cancer,

EVERY TUMOR
HAS AN ESCAPE PLAN

Endocrine therapy can help corner ER+ advanced breast cancer

Endocrine therapy is a cornerstone of treatment for ER+ patients.[1] In fact, approximately 60% of breast cancers are ER+ and initially respond to endocrine therapy.[2,3] However, all patients eventually develop resistance—a sign that their disease is progressing.[1,3,4]

Activation of the PI3K/Akt/mTOR pathway is one way through which ER+ advanced breast cancer circumvents endocrine therapy

Hyperactivation of the PI3K/Akt/mTOR pathway can provide a way out for ER+ breast cancer cells, which can lead to resistance.[5] And this resistance may ultimately result in estrogen-independent disease progression.[4]
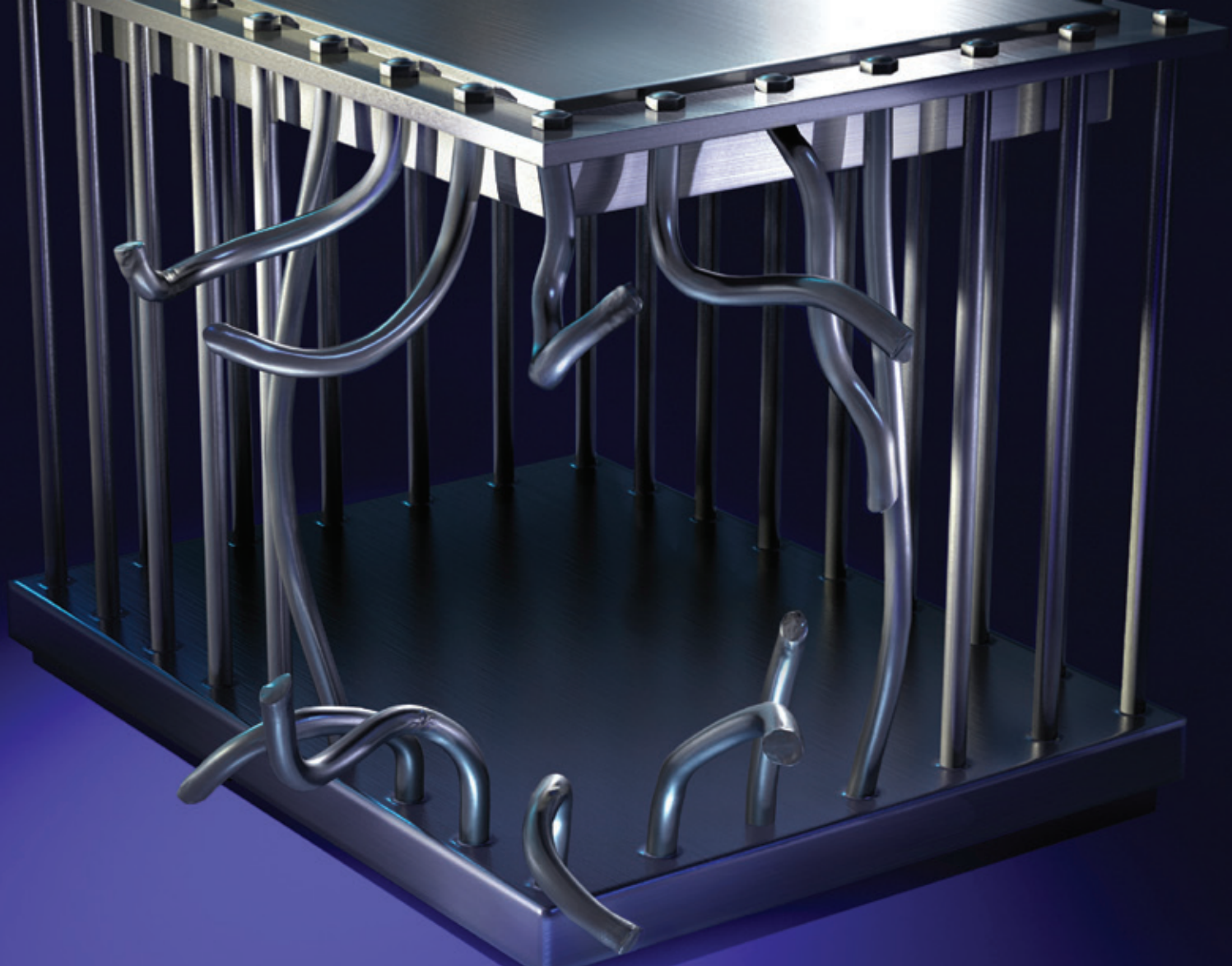
NOVARTIS
ONCOLOGY

We're looking for ways to help block these escape paths

Novartis is committed to advancing research into the PI3K/Akt/mTOR pathway to help find new options for patients with cancer.

For more information on the role of the PI3K/Akt/mTOR signaling pathway in endocrine resistance and disease progression, please visit www.tumorescape.com.

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Interested in contributing to *TargetedTherapies in Cancer*?
If you'd like to submit an article for consideration in an upcoming issue, please e-mail Devera Pine at dpine@onclive.com.

From the Publisher

The use of targeted therapies for the treatment of cancer is one of the most rapidly changing areas of oncology, with new therapies and combination strategies making inroads into current standards of care. As therapeutics continue to emerge on the market at a record-breaking pace, the question of how to most effectively apply them—and how to optimize outcomes in patients—becomes ever more pressing. For community oncologists, the challenge is staying up to date, both on clinical trial evidence and on emerging strategies for applying targeted therapies to patient care.

The International Journal of Targeted Therapies in Cancer is a quarterly, peer-reviewed publication designed to help community oncologists integrate information about targeted therapies quickly and efficiently. Our goal is to help oncology healthcare professionals gain a greater understanding of new therapies and diagnostics—and then to provide the latest evidence-based strategies for applying these to clinical practice, ultimately improving outcomes for patients.

With that in mind, on behalf of *The International Journal of Targeted Therapies in Cancer*, welcome. We invite you to submit manuscripts about the emerging area of targeted therapies, biomarkers, and diagnostic testing that are relevant and applicable to community oncologists, and we invite you also to let us know what you think.

—Peter Ciszewski
PUBLISHER

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Maurie Markman, MD

Pharmacogenomics in Action

Breast Cancer and the Intricacies of Applying Genotyping to the Treatment of Patients

> It has been difficult to confirm the utility resulting from knowledge of unique genetic factors existing within the patient.

Recently reported and truly impressive clinical trial data have revealed the relevance of administering antineoplastic agents specifically and prospectively targeted to molecular abnormalities within the cancers of individual patients. The justification for such an approach is based both on preclinical and clinical data that have revealed the relevance of the particular target in the disease setting.

Less frequently discussed, but potentially equally important in optimizing the ultimate efficacy and minimizing the toxicity of a therapeutic strategy, are genetically defined features within the individual patient's normal, rather than tumor, molecular environment. Unfortunately, while this concept is attractive, it has been difficult to confirm the utility resulting from knowledge of unique genetic factors existing within the patient, even in settings where the clinical relevance of such relationships has been proposed based on solid preclinical or epidemiological considerations.

A prominent example of this situation is the intense investigation and surrounding controversy associated with a potential role for genetic variants of cytochrome P450 (CYP2D6) in influencing the metabolic fate and ultimate biological activity and clinical effectiveness of tamoxifen in the management of breast cancer. Studies from excellent investigative groups both supporting and rebutting the relevance of such genetic variation have been reported in the peer-reviewed literature.[1] At the present time, it remains uncertain whether patients who poorly metabolize the parent compound to its active metabolites are less likely to achieve a favorable outcome compared with individuals who have the genetically defined apparatus to metabolize this agent extensively.

And, while the prognostic influence of the existence of specific cytochrome P450 genetic variants in defining the benefits of tamoxifen therapy in individual patients remains unclear, an even more unsettled question is the issue of whether pretherapy knowledge of the presence of such variations in a patient can be utilized to modify the planned therapy to favorably influence that outcome.

In a most provocative report designed to begin to address this issue, investigators genotyped 119 women receiving tamoxifen for a minimum of 4 months for the management of their breast cancer.[2] The inclusion criteria also required that participants were not taking any medications known to inhibit CYP2D6.

Patients found to be genetically defined as intermediate or poor metabolizers of tamoxifen had the daily oral dose of this agent increased from 20 mg to 40 mg. (Of note, it is recognized that this higher dose of tamoxifen can be administered safely in this clinical setting.) Individuals identified as being extensive metabolizers had their dose remain at 20 mg per day. ●

Maurie Markman, MD, is senior vice president for clinical affairs and national director for medical oncology at Cancer Treatment Centers of America, Eastern Regional Medical Center, Philadelphia, Pennsylvania.
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IN ADVANCED PROSTATE CANCER...

PROVENGE

ACTIVATE THE POWER OF THE IMMUNE SYSTEM. EXTEND SURVIVAL.

Resting T cell

PROVENGE

PROVENGE-activated T cell

Activated T cell attacks prostate cancer

Prostate cancer cell
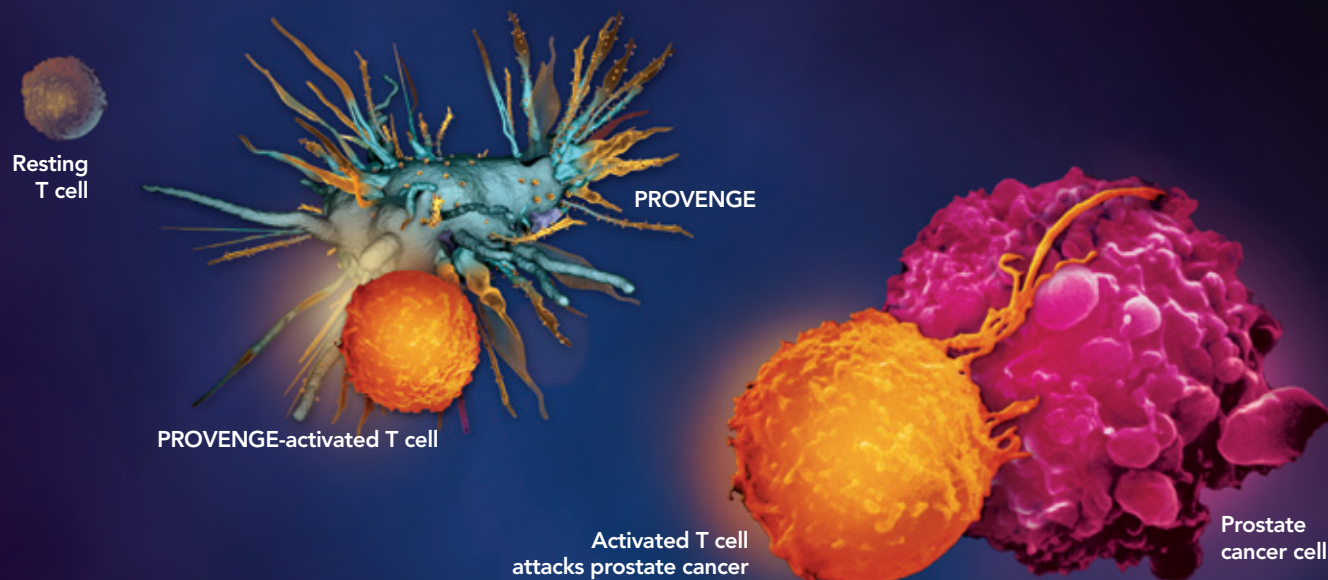
- **PROVENGE** extends median survival beyond 2 years[1]
- Only 1.5% of patients treated with **PROVENGE** in the pivotal trial discontinued treatment due to adverse events[2]
  — The most common adverse events in **PROVENGE** trials were chills, fatigue, fever, back pain, nausea, joint ache, and headache[2]
- **PROVENGE** is the first and only FDA-approved immunotherapy for advanced prostate cancer
- The NCCN recommends **PROVENGE** as a *first-line* treatment for men with asymptomatic or minimally symptomatic metastatic castrate resistant prostate cancer *(NCCN Category 1 recommendation)*[3]

INDICATION: PROVENGE® (sipuleucel-T) is an autologous cellular immunotherapy indicated for the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer.

IMPORTANT SAFETY INFORMATION: PROVENGE is intended solely for autologous use and is not routinely tested for transmissible infectious diseases. In controlled clinical trials, serious adverse events reported in the PROVENGE group include acute infusion reactions (occurring within 1 day of infusion) and cerebrovascular events. Severe (Grade 3) acute infusion reactions were reported in 3.5% of patients in the PROVENGE group. Reactions included chills, fever, fatigue, asthenia, dyspnea, hypoxia, bronchospasm, dizziness, headache, hypertension, muscle ache, nausea, and vomiting. No Grade 4 or 5 acute infusion reactions were reported in patients in the PROVENGE group.

The most common adverse events (incidence ≥15%) reported in the PROVENGE group were chills, fatigue, fever, back pain, nausea, joint ache, and headache.

For more information on PROVENGE, please see Brief Summary of Prescribing Information on adjacent page.

Dendreon
Targeting Cancer, Transforming Lives®

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PROVENGE®
(sipuleucel-T)

PROVENGE® (sipuleucel-T)
Suspension for Intravenous Infusion   **Rx Only**

BRIEF SUMMARY – See full Prescribing Information for complete product information

INDICATIONS AND USAGE: PROVENGE® (sipuleucel-T) is an autologous cellular immunotherapy indicated for the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer.

DOSAGE AND ADMINISTRATION
- **For Autologous Use Only.**
- The recommended course of therapy for PROVENGE is 3 complete doses, given at approximately 2-week intervals.
- Premedicate patients with oral acetaminophen and an antihistamine such as diphenhydramine.
- Before infusion, confirm that the patient's identity matches the patient identifiers on the infusion bag.
- **Do Not Initiate Infusion of Expired Product.**
- Infuse PROVENGE intravenously over a period of approximately 60 minutes. **Do Not Use a Cell Filter.**
- Interrupt or slow infusion as necessary for acute infusion reactions, depending on the severity of the reaction.

(See Dosage and Administration [2] of full Prescribing Information.)

CONTRAINDICATIONS: None.

WARNINGS AND PRECAUTIONS

- **PROVENGE is intended solely for autologous use.**

- **Acute infusion reactions** (reported within 1 day of infusion) included, but were not limited to, fever, chills, respiratory events (dyspnea, hypoxia, and bronchospasm), nausea, vomiting, fatigue, hypertension, and tachycardia. In controlled clinical trials, 71.2% of patients in the PROVENGE group developed an acute infusion reaction.

  In controlled clinical trials, severe (Grade 3) acute infusion reactions were reported in 3.5% of patients in the PROVENGE group. Reactions included chills, fever, fatigue, asthenia, dyspnea, hypoxia, bronchospasm, dizziness, headache, hypertension, muscle ache, nausea, and vomiting. The incidence of severe events was greater following the second infusion (2.1% vs 0.8% following the first infusion), and decreased to 1.3% following the third infusion. Some (1.2%) patients in the PROVENGE group were hospitalized within 1 day of infusion for management of acute infusion reactions. No Grade 4 or 5 acute infusion reactions were reported in patients in the PROVENGE group.

  Closely monitor patients with cardiac or pulmonary conditions. In the event of an acute infusion reaction, the infusion rate may be decreased, or the infusion stopped, depending on the severity of the reaction. Appropriate medical therapy should be administered as needed.

- **Handling Precautions for Control of Infectious Disease.** PROVENGE is **not** routinely tested for transmissible infectious diseases. Therefore, patient leukapheresis material and PROVENGE may carry the risk of transmitting infectious diseases to health care professionals handling the product. Universal precautions should be followed.

- **Concomitant Chemotherapy or Immunosuppressive Therapy.** Use of either chemotherapy or immunosuppressive agents (such as systemic corticosteroids) given concurrently with the leukapheresis procedure or PROVENGE has not been studied. PROVENGE is designed to stimulate the immune system, and concurrent use of immunosuppressive agents may alter the efficacy and/or safety of PROVENGE. Therefore, patients should be carefully evaluated to determine whether it is medically appropriate to reduce or discontinue immunosuppressive agents prior to treatment with PROVENGE.

- **Product Safety Testing.** PROVENGE is released for infusion based on the microbial and sterility results from several tests: microbial contamination determination by Gram stain, endotoxin content, and in-process sterility with a 2-day incubation to determine absence of microbial growth. The final (7-day incubation) sterility test results are not available at the time of infusion. If the sterility results become positive for microbial contamination after PROVENGE has been approved for infusion, Dendreon will notify the treating physician. Dendreon will attempt to identify the microorganism, perform antibiotic sensitivity testing on recovered microorganisms, and communicate the results to the treating physician. Dendreon may request additional information from the physician in order to determine the source of contamination.

(See Warnings and Precautions [5] of full Prescribing Information.)

ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety evaluation of PROVENGE is based on 601 prostate cancer patients in the PROVENGE group who underwent at least 1 leukapheresis procedure in four randomized, controlled clinical trials. The control was non-activated autologousperipheral blood mononuclear cells.

The most common adverse events, reported in patients in the PROVENGE group at a rate ≥15%, were chills, fatigue, fever, back pain, nausea, joint ache, and headache. Severe (Grade 3) and life-threatening (Grade 4) adverse events were reported in 23.6% and 4.0% of patients in the PROVENGE group compared with 25.1% and 3.3% of patients in the control group. Fatal (Grade 5) adverse events were reported in 3.3% of patients in the PROVENGE group compared with 3.6% of patients in the control group.

Serious adverse events were reported in 24.0% of patients in the PROVENGE group and 25.1% of patients in the control group. Serious adverse events in the PROVENGE group included acute infusion reactions *(see Warnings and Precautions)*, cerebrovascular events, and single case reports of eosinophilia, rhabdomyolysis, myasthenia gravis, myositis, and tumor flare.

PROVENGE was discontinued in 1.5% of patients in Study 1 (PROVENGE group n=341; Control group n=171) due to adverse events. Some patients who required central venous catheters for treatment with PROVENGE developed infections, including sepsis. A small number of these patients discontinued treatment as a result. Monitoring for infectious sequelae in patients with central venous catheters is recommended.

Each dose of PROVENGE requires a standard leukapheresis procedure approximately 3 days prior to the infusion. Adverse events that were reported ≤1 day following a leukapheresis procedure in ≥5% of patients in controlled clinical trials included citrate toxicity (14.2%), oral paresthesia (12.6%), paresthesia (11.4%), and fatigue (8.3%).

Table 1 provides the frequency and severity of adverse events reported in ≥5% of patients in the PROVENGE group of randomized, controlled trials of men with prostate cancer. The population included 485 patients with metastatic castrate resistant prostate cancer and 116 patients with non-metastatic androgen dependent prostate cancer who were scheduled to receive 3 infusions of PROVENGE at approximately 2-week intervals. The population was age 40 to 91 years (median 70 years), and 90.6% of patients were Caucasian.

Table 1 Incidence of Adverse Events Occurring in ≥5% of Patients Randomized to PROVENGE

	PROVENGE (N = 601)		Control* (N = 303)	
	All Grades n (%)	**Grade 3-5 n (%)**	**All Grades n (%)**	**Grade 3-5 n (%)**
Any Adverse Event	**591 (98.3)**	**186 (30.9)**	**291 (96.0)**	**97 (32.0)**
Chills	319 (53.1)	13 (2.2)	33 (10.9)	0 (0.0)
Fatigue	247 (41.1)	6 (1.0)	105 (34.7)	4 (1.3)
Fever	188 (31.3)	6 (1.0)	29 (9.6)	3 (1.0)
Back pain	178 (29.6)	18 (3.0)	87 (28.7)	9 (3.0)
Nausea	129 (21.5)	3 (0.5)	45 (14.9)	0 (0.0)
Joint ache	118 (19.6)	11 (1.8)	62 (20.5)	5 (1.7)
Headache	109 (18.1)	4 (0.7)	20 (6.6)	0 (0.0)
Citrate toxicity	89 (14.8)	0 (0.0)	43 (14.2)	0 (0.0)
Paresthesia	85 (14.1)	1 (0.2)	43 (14.2)	0 (0.0)
Vomiting	80 (13.3)	2 (0.3)	23 (7.6)	0 (0.0)
Anemia	75 (12.5)	11 (1.8)	34 (11.2)	7 (2.3)
Constipation	74 (12.3)	1 (0.2)	40 (13.2)	3 (1.0)
Pain	74 (12.3)	7 (1.2)	20 (6.6)	3 (1.0)
Paresthesia oral	74 (12.3)	0 (0.0)	43 (14.2)	0 (0.0)
Pain in extremity	73 (12.1)	5 (0.8)	40 (13.2)	1 (0.3)
Dizziness	71 (11.8)	2 (0.3)	34 (11.2)	0 (0.0)
Muscle ache	71 (11.8)	3 (0.5)	17 (5.6)	0 (0.0)
Asthenia	65 (10.8)	6 (1.0)	20 (6.6)	2 (0.7)
Diarrhea	60 (10.0)	1 (0.2)	34 (11.2)	3 (1.0)
Influenza-like illness	58 (9.7)	0 (0.0)	11 (3.6)	0 (0.0)
Musculoskeletal pain	54 (9.0)	3 (0.5)	31 (10.2)	3 (1.0)
Dyspnea	52 (8.7)	11 (1.8)	14 (4.6)	3 (1.0)
Edema peripheral	50 (8.3)	1 (0.2)	31 (10.2)	1 (0.3)
Hot flush	49 (8.2)	2 (0.3)	29 (9.6)	1 (0.3)
Hematuria	46 (7.7)	6 (1.0)	18 (5.9)	3 (1.0)
Muscle spasms	46 (7.7)	2 (0.3)	17 (5.6)	0 (0.0)

Table 1 Incidence of Adverse Events Occurring in ≥5% of Patients Randomized to PROVENGE

	PROVENGE (N = 601)		Control* (N = 303)	
	All Grades n (%)	Grade 3-5 n (%)	All Grades n (%)	Grade 3-5 n (%)
Hypertension	45 (7.5)	3 (0.5)	14 (4.6)	0 (0.0)
Anorexia	39 (6.5)	1 (0.2)	33 (10.9)	3 (1.0)
Bone pain	38 (6.3)	4 (0.7)	22 (7.3)	3 (1.0)
Upper respiratory tract infection	38 (6.3)	0 (0.0)	18 (5.9)	0 (0.0)
Insomnia	37 (6.2)	0 (0.0)	22 (7.3)	1 (0.3)
Musculoskeletal chest pain	36 (6.0)	2 (0.3)	23 (7.6)	2 (0.7)
Cough	35 (5.8)	0 (0.0)	17 (5.6)	0 (0.0)
Neck pain	34 (5.7)	3 (0.5)	14 (4.6)	2 (0.7)
Weight decreased	34 (5.7)	2 (0.3)	24 (7.9)	1 (0.3)
Urinary tract infection	33 (5.5)	1 (0.2)	18 (5.9)	2 (0.7)
Rash	31 (5.2)	0 (0.0)	10 (3.3)	0 (0.0)
Sweating	30 (5.0)	1 (0.2)	3 (1.0)	0 (0.0)
Tremor	30 (5.0)	0 (0.0)	9 (3.0)	0 (0.0)

*Control was non-activated autologous peripheral blood mononuclear cells.

Cerebrovascular Events. In controlled clinical trials, cerebrovascular events, including hemorrhagic and ischemic strokes, were reported in 3.5% of patients in the PROVENGE group compared with 2.6% of patients in the control group.

(See Adverse Reactions [6] of full Prescribing Information.)

To report SUSPECTED ADVERSE REACTIONS, contact Dendreon Corporation at 1-877-336-3736 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

Dendreon Corporation
Seattle, Washington 98101

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This illustration depicts the process of tumor angiogenesis.
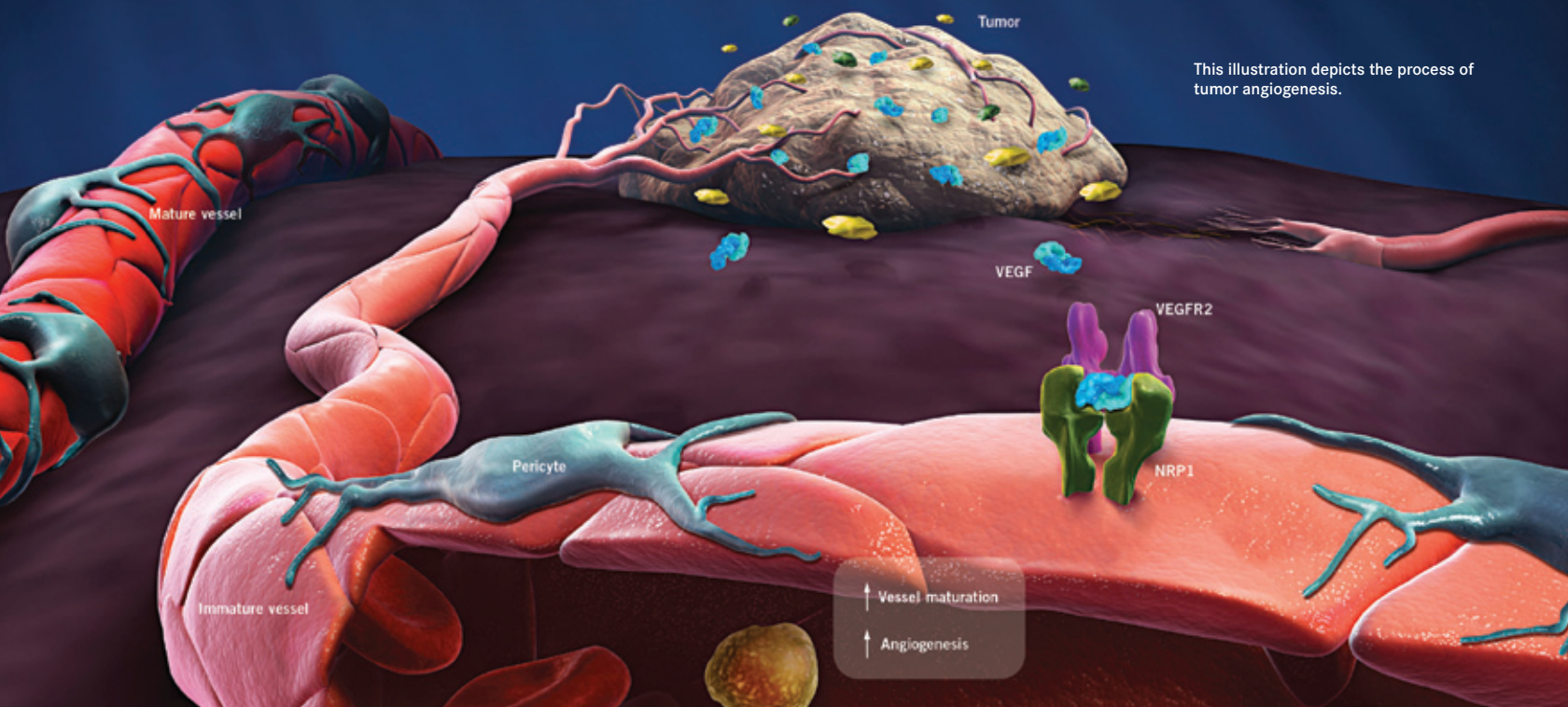
Targeted Therapies

Do Cell Signaling Pathways Have a Place in Clinical Practice?

By Jane de Lartigue, PhD

From the days when cancer was thought to arise from foreign viral genes to today, when novel therapies are rapidly translated from bench to bedside, it is sometimes difficult to grasp how far we've come. It was only some 30 years ago, in the late 1970s, when researchers and clinicians witnessed the beginnings of a revolution in cancer research with the discovery that mutations in our genes—particularly those that govern cell signaling—were in fact responsible for tumorigenesis.

Robert A. Weinberg, PhD, director of the Ludwig Center for Molecular Oncology at the Massachusetts Institute of Technology and co-author of the seminal paper on cancer and cell signaling, "The Hallmarks of Cancer," and the recently published "Hallmarks of Cancer: The Next Generation," has been intimately involved in this revolution. He suggested that the first solid evidence linking the signaling pathways in our cells to cancer stemmed from the Nobel Prize–winning discovery (by J. Michael Bishop and Harold E. Varmus) of *SRC* as the first proto-oncogene (a normal gene that can drive cancer when mutated), and the demonstration by Tony Hunter that the SRC protein is a tyrosine kinase involved in cell signaling. "Much of what followed derived in one way or another from that finding," said Weinberg.

Cell Communication and Cancer

Researchers are now aware of the existence of at least 50 genes with clear oncogenic function and, thus far, every major signaling pathway has been implicated in cancer.

Cells communicate with one another and respond to their environment predominantly by means of chemical signaling molecules that bind extracellular receptors on the surface or diffuse into the cell to bind internal receptors. This process stimulates a cascade of proteins that amplify signals and deliver them to intracellular destinations, where they mediate changes in cellular activity.

Cancer cells are characterized by their ability to promote proliferation and growth, inhibit cell death, evoke angiogenesis, and, eventually, colonize other parts of the body through invasion and migration. Each of these processes is regulated by cell signaling pathways, and many genetic abnormalities found in cancer involve signaling proteins. These abnormalities result in the downregulation of pathways that oppose the above behaviors and upregulation of pathways that promote them.

Tyrosine Kinases and Cancer
In his book *The Emperor of All Maladies*, Siddhartha Mukherjee discusses the way in which researchers began tracing what he calls the "slow march of cancer from one gene mutation to the next." One of the most important factors in that process is tyrosine kinase.

Tyrosine kinases are enzymes that phosphorylate tyrosine residues in target proteins, stimulating or inhibiting their activity **(see article, page 14)**. The majority are cell surface receptor tyrosine kinases (RTKs), activated by growth factors, cytokines, and hormones.

As key regulators of proliferation and growth, more than 30 RTKs have been implicated in cancer, as have the 2 main signaling pathways that they regulate—the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathways.

The *RAS* gene is central to the MAPK pathway: Activating mutations of the gene are found in 20% to 25% of all human tumors and 90% of pancreatic cancers. A critical direct effector of the Ras protein in mammalian cells is the serine threonine kinase Raf-1, and activating mutations of one of the Raf isoforms, B-raf, have been found in >60% of human malignant melanomas, as well as in some colon, thyroid, and lung tumors. According to Weinberg, who is also a member of the Whitehead Institute for Biomedical Research and is best known for his discoveries of the human oncogene *RAS* and the tumor suppressor retinoblastoma protein, the receptor/Ras/MAPK pathway is not only the most studied, but also appears to be most central to driving the runaway proliferation of cancer cells.

The PI3K pathway is activated by both RTKs and Ras, and, in turn, activates a plethora of downstream signaling pathways through the generation of the lipid second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3). A key target of PI3K is Akt (also known as protein kinase B), which itself has at least 13 known substrates in mammalian cells involved in growth, apoptosis, cell cycle control, and protein synthesis. Every member of this signaling axis is frequently altered in cancer; estimates suggest that these alterations account for up to 30% of all human cancers.

Other Key Pathways
In addition to driving pro-growth signaling pathways, cancer mutations can lead to inhibition of antiproliferative pathways. The best understood of these is the tumor growth factor β (TGFβ) pathway. The Wnt/β-catenin pathway is also important, with a mutation in the adenomatous polyposis coli (APC) protein in this pathway found in 90% of colon cancers. Subsequently, cancer mutations have also been found to occur in signaling pathways involved in regulating cell death, angiogenesis, invasion, migration, and the epithelial-to-mesenchymal transition (EMT) characteristic of tumor metastasis, a process responsible for 90% of all cancer deaths.

Apoptotic pathways implicated in cancer include the death and survival factor pathways, among them Fas ligand (FasL), tumor necrosis factor α (TNFα), and numerous growth factor pathways. The vast majority of mutations occur in the p53 protein. Commonly affected angiogenic and EMT-related pathways are the vascular endothelial growth factor (VEGF), hypoxia, and integrin signaling pathways, as well as the Wnt, TGF, and RTK-regulated pathways.

New Options for Treating Patients
Research that began with the discovery of the first oncogene has now led to the emergence of a new generation of cancer therapies that specifically target cell signaling molecules. Among them are a number of small molecule inhibitors and monoclonal antibodies that work against the kinases.

Currently, more than 20 monoclonal antibodies are approved by the FDA, the best known of which is trastuzumab (Herceptin), which has had a major impact on the treatment of HER2-positive metastatic breast cancer. Other examples include bevacizumab (Avastin), cetuximab (Erbitux), and panitumumab (Vectibix). Among the FDA-approved kinase inhibitors are dasatinib (Sprycel), erlotinib (Tarceva), gefitinib (Iressa), imatinib (Gleevec), lapatinib (Tykerb), nilotinib (Tasigna), sorafenib (Nexavar), and sunitinib (Sutent). These drugs target a host of signaling proteins ranging from the epithelial growth factor receptor (EGFR), an RTK, to abl, a tyrosine kinase involved in cell cycle control.

Treatment Challenges
Because cancer cells commonly have more than 1 mutation, the development of resistance to these drugs represents an ongoing challenge. Current thinking suggests that the model of linear cell signaling pathways should be replaced by one incorporating large, complex signaling networks in which cancer genes are often enriched in signaling "hubs."

> *Current thinking suggests that the model of linear cell signaling pathways should be replaced by one incorporating large, complex signaling networks in which cancer genes are often enriched in signaling "hubs."*

"Over the next decade the field is heading toward understanding how these 'canonical' pathways intercommunicate and function in a combinatorial fashion to create cancer cell phenotypes," noted Weinberg. Indeed, clinical research is already focused on examining the synergy between combinations of drugs targeting multiple pathways.

Jane de Lartigue, PhD, is a freelance medical writer and editor based in the United Kingdom.
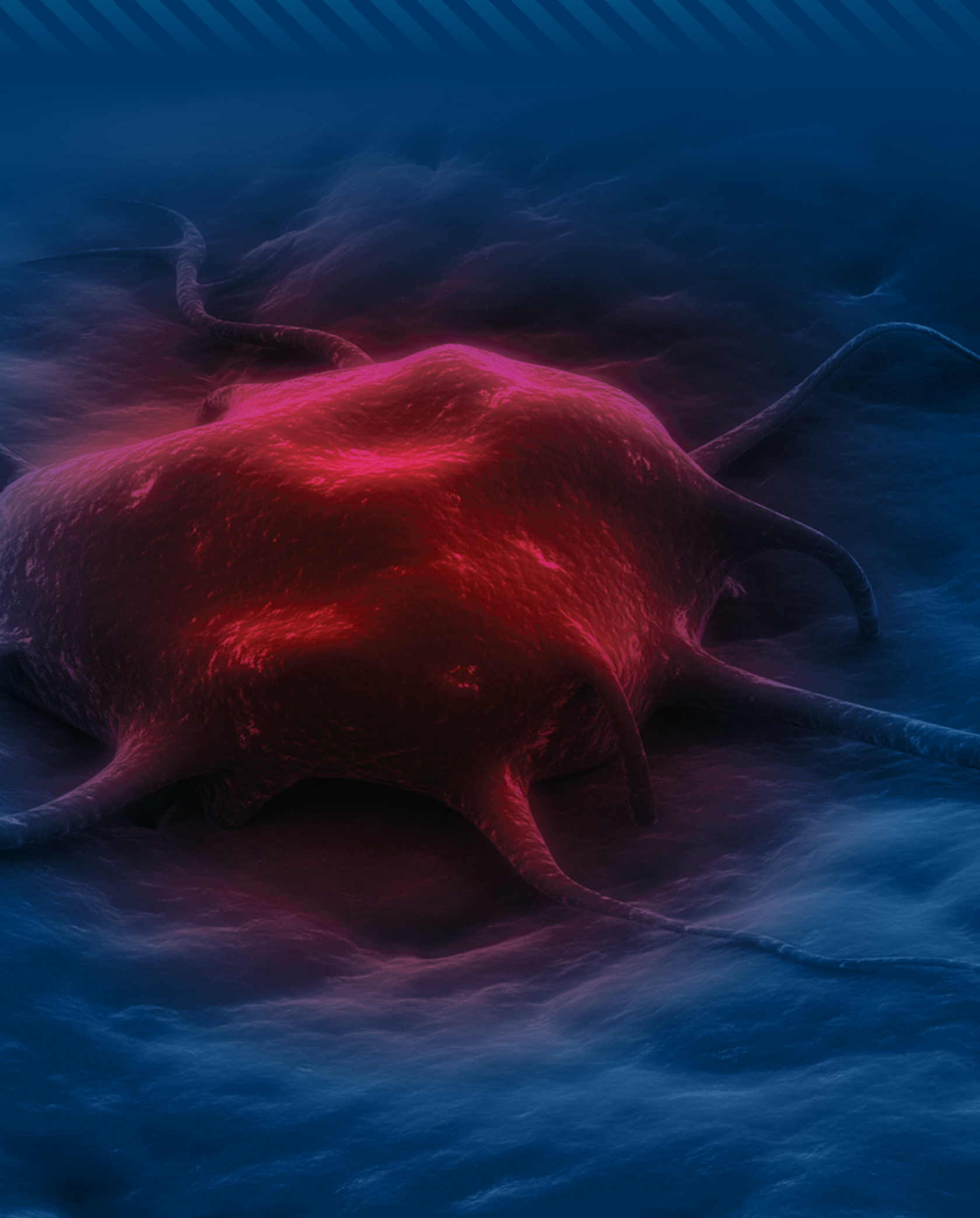
What Turns a Good Cell Bad?

Why New Theories on the "Hallmarks of Cancer" Matter to Community Oncologists

By Jane de Lartigue, PhD

It has been more than a decade since Douglas Hanahan, PhD, and Robert A. Weinberg, PhD, published their seminal review of cancer, which outlined 6 essential alterations in cell physiology that govern the transformation of normal cells into malignant tumors. Since then, it has become one of the most cited articles of all time, reflecting the widespread acceptance of these "hallmarks" of cancer.

In light of the recently published update of this review, we reflect on our current understanding of the biology of tumors and the signaling pathways that cancer cells use to achieve these hallmarks, whether the hallmarks are still widely applicable, and how they guide both cancer research and therapeutic strategies for the community oncologist.

Defining 6 Hallmarks of Cancer

Hanahan and Weinberg initially outlined 6 hallmarks that they believed were essential to the transformation of normal cells into malignant cancer cells in most, if not all, human cancers:

1. Self-Sustained Growth
2. Avoiding Growth Inhibition

Normal cells need to receive growth signals before they can begin to grow and divide, and their growth is kept in check by a number of antigrowth signals. Cancer cells acquire the ability to essentially drive through a red light, bypassing the requirement for growth signals and avoiding antigrowth signals; this forms the basis of the first 2 hallmarks.

There are several ways in which cancer cells can stimulate their own growth. They can alter extracellular growth signals, either by stimulating the normal cells in their surrounding environment to produce growth factors, or by producing growth factors to which they themselves are responsive.

They can modify the expression of the cell surface receptors that transduce these growth signals, the most prominent example being the tyrosine kinase receptors, such as epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2), both commonly overexpressed in many different kinds of cancer.

Finally, they can alter the intracellular signaling networks that translate growth signals into action, via overexpression of components of pathways that stimulate growth or defects in feedback mechanisms that attenuate growth signaling. The PI3K/Akt and Ras/Raf/mitogen-activated protein kinase (MAPK) pathways play a key role in this respect, reflected in the fact that the PI3K/Akt pathway is one of the most perturbed in human cancer, and that a quarter of all cancers have Ras alterations.

Antigrowth signals are primarily channeled through 2 "gatekeeper" proteins: retinoblastoma protein (pRb) and p53. Cancer cells further promote their own growth by disrupting the function of these 2 proteins, which in normal cells control transcription factors that regulate the expression of growth-related genes.

3. Avoiding Death
Efficient disposal of defective cells through programmed cell death (apoptosis) is an integral part of the normal function of multicellular organisms.

The cellular machinery that coordinates apoptosis is divided into 2 classes: sensors that detect "survival" and "death" signals in the environment (including the insulin-like growth factor receptor [IGFR] and the FAS receptor) and effectors that either elicit or suppress apoptosis in response to those signals (including p53 and members of the Bcl-2 family).

Cancer cells acquire the ability to avoid apoptosis, the third hallmark, in a number of ways. The most common is a loss of p53, which normally initiates apoptosis, and is lost in more than 50% of human cancers. Other mechanisms include increased expression of antiapoptotic Bcl-2 family members or of the IGFR, and decreased expression of the FAS receptor.

4. Limitless Division

Surprisingly, acquiring these first 3 hallmarks is not sufficient for unlimited cell growth within tumors. Normal cells also have a finite potential to divide. After a certain number of divisions, shortening of the telomeres, which protect the ends of the chromosomes, prevents the cell from further dividing to avoid chromosomal damage.

Hesed Padilla-Nash and Thomas Ried/NIGMS Image Gallery
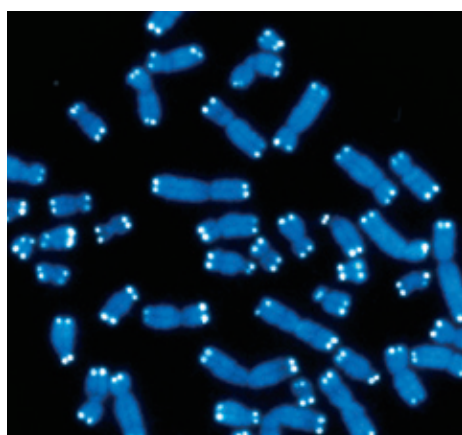
White pinpoints display telomeres in the 46 human chromosomes, shown in blue.

In order to grow unchecked, cancer cells have to develop the ability to multiply without limit. Approximately 85% to 90% of cancer cells achieve this end by upregulating the expression of the enzyme telomerase, which helps to maintain the telomeres and prevent their shortening.

5. Stimulating Angiogenesis

Normal cells must typically reside within 100 μm of a blood vessel in order to obtain necessary oxygen and nutrients. This limits the ability of cells to invade all areas of the body and of tissues to grow beyond a certain size. In order to overcome this issue, cancer cells acquire a fifth hallmark, the ability to stimulate angiogenesis (growth of new blood vessels).

In normal cells, the balance between proangiogenic and antiangiogenic signaling pathways is regulated by an "angiogenic switch," which is tightly regulated in normal cells so that angiogenesis is only turned on during processes such as wound healing. In contrast, in cancer cells it is almost always turned on, stimulating the growth of new blood vessels and sustaining tumor growth.

Among the best-known proangiogenic pathways is the vascular endothelial growth factor (VEGF) pathway.

6. Invasion and Metastasis

The final hallmark is the key to the destructive capability of tumors and is responsible for 90% of all cancer-related deaths. It is also the hallmark of which we currently have the poorest understanding.

Some cancer cells are able to break away from the original tumor, migrate to a new area of the body, and establish a second site of tumor formation (metastasis). In order to do this successfully, among other characteristics, cancer cells have to be able to reduce cell-to-cell adhesion and increase cell motility. The most common alteration in cancer cells resulting in invasion and metastasis is in the tethering protein E-cadherin.

Employing the Hallmarks in Cancer Therapy

In the past decade there has been a continuing expansion of our anticancer armamentarium. To date, rather than targeting hallmarks as a whole, most new drugs have been developed to target specific proteins within key hallmark-associated signaling pathways.

The most promising advances have come from the development of small-molecule inhibitors and monoclonal antibodies targeting the tyrosine kinase receptors. These include the EGFR inhibitor gefitinib (Iressa, AstraZeneca) and the HER2 monoclonal antibody trastuzumab (Herceptin, Genentech), which primarily target the hallmark of unrestricted growth.

The first clinical validation that inhibiting a tumor's blood supply would thwart its growth—the angiogenesis hallmark—came in 2003 when bevacizumab (Avastin, Genentech/Roche), an anti-VEGF monoclonal antibody, proved effective against colorectal cancer. Although the agent has been controversial in the treatment of breast cancer, the drug remains FDA-approved for 4 other cancer types.

Agents targeting VEGF receptors are a robust area of exploration. One prominent example is axitinib (Pfizer Inc), which the FDA is now reviewing as a treatment for renal cell carcinoma.

Drugs in development that focus on the hallmark of unlimited cell division include imetelstat (GRN163L, Geron). The short-chain nucleic acid molecule, currently in phase II clinical trials, has displayed inhibitory activity against telomerase.

Research in the area of the sixth hallmark, invasion and metastasis, has exploded in recent years, and targeted therapies have now begun to emerge. A prime example is inhibitors of the hepatocyte growth factor (HGF) receptor, c-MET, which has a key role in cell motility and invasion. A number of these agents are in development, including tivantinib (ARQ 197, ArQule), currently in the final phase of clinical development for the treatment of non-small cell lung cancer.

Targeting tumor-suppressor proteins, such as p53, has proved much more challenging since they often defy conventional drug development paradigms. However, it has driven innovative new drug design processes and the development of treatments such as vaccines and gene therapy.

In spite of these substantial therapeutic advances, shutting off a single pathway may not be sufficient to halt tumorigenesis, since other pathways may simply take over to drive hallmark acquisition, reflected in the level of resistance to these therapies.

Much research is already focused on the idea of targeting multiple different pathways with combinations of targeted agents. However, we are also beginning to move away from our view of tumors as homogenous

Shutting off a single pathway may not be sufficient to halt tumorigenesis.
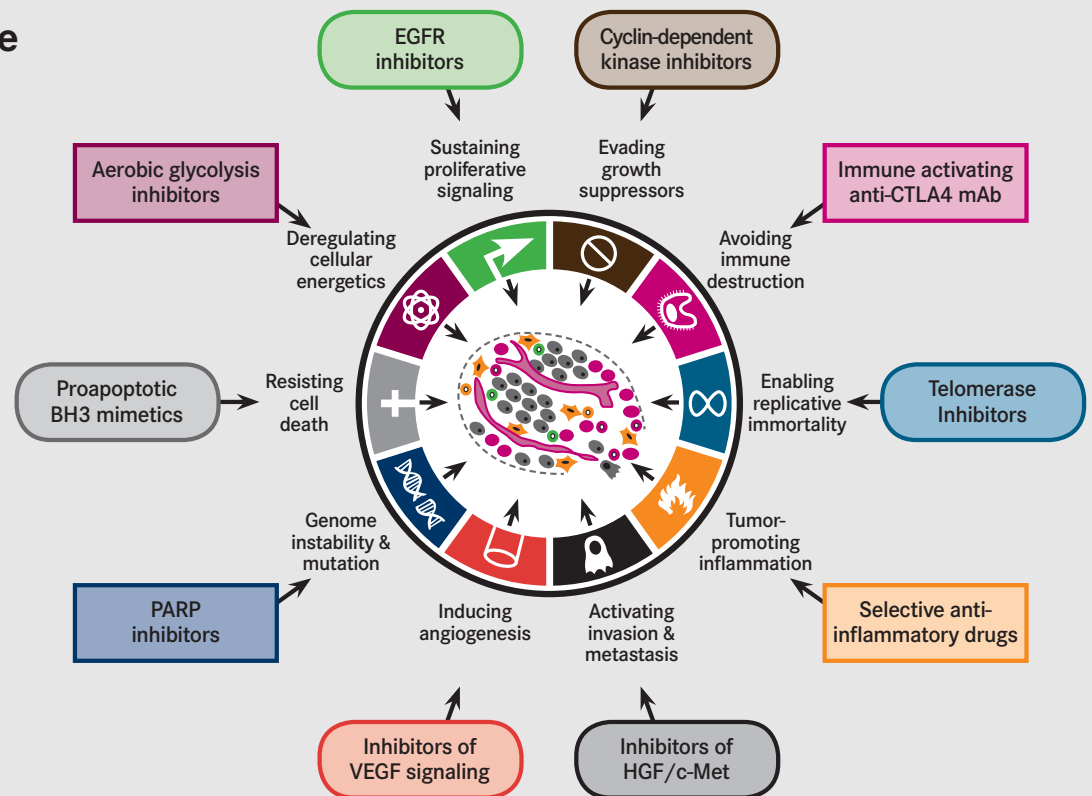
Strategies Targeting the Hallmarks of Cancer

This figure illustrates some of the many approaches employed in developing therapeutics targeted to the known and emerging hallmarks of cancer.

EGFR indicates epidermal growth factor receptor; CTLA4, cytotoxic T lymphocyte-associated antigen 4; mAb, monoclonal antibody; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; PARP, poly-(ADP ribose) polymerase.

Source: Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646-674. Reprinted with permission.

EGFR inhibitors

Cyclin-dependent kinase inhibitors

Aerobic glycolysis inhibitors

Immune activating anti-CTLA4 mAb

Sustaining proliferative signaling

Evading growth suppressors

Avoiding immune destruction

Deregulating cellular energetics

Proapoptotic BH3 mimetics

Resisting cell death

Enabling replicative immortality

Telomerase Inhibitors

PARP inhibitors

Genome instability & mutation

Tumor-promoting inflammation

Selective anti-inflammatory drugs

Inducing angiogenesis

Activating invasion & metastasis

Inhibitors of VEGF signaling

Inhibitors of HGF/c-Met

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collections of cells that are all at the same stage. Different cells within the tumor population may have acquired different hallmarks by separate mechanisms, and therefore co-targeting multiple components of different hallmark processes may also be beneficial.

New Hallmarks Versus a New View of Cancer

Another shift that is occurring is the identification of other potential hallmarks; researchers have proposed a number of other characteristics that appear to be common to all tumor cells, and that the 6 hallmarks are not sufficient for tumorigenesis.

Two of the most commonly proposed additional hallmarks are evasion of the immune system (tumors are able to subvert the normal immune response or develop defensive responses to it) and reprogramming of cell metabolism (the so-called Warburg effect, whereby tumors are able to reprogram their glucose metabolism, using glycolysis in the presence of oxygen).

It has also been suggested that there are "enabling characteristics," which must first occur in order for cancer cells to be able to begin acquiring hallmarks. Most prominent is the development of genomic instability in cancer cells. In normal cells, mutation is a rare event since it is detected by genomic "guardians," which drive cells to either repair the damage or undergo apoptosis. In order to accumulate mutations, cancer cells must remove these protective mechanisms, allowing the unchecked generation of mutations that drive hallmark acquisition.

The second proposed enabling characteristic is tumor-promoting inflammation. The body's response to a tumor is to elicit an immune response, causing inflammation. Recently, it has been revealed that cancer cells may use this inflammatory response to their benefit. Persistent inflammation in the surrounding environment inadvertently provides the tumor with a source of growth, survival, and proangiogenic factors, enabling the development of several hallmarks.

The place for hallmarks and their enabling characteristics has led to some debate. Some believe that tumorigenesis is vastly more complex and that there are many more hallmarks than currently described. Others believe that only metastasis is the key defining hallmark of cancer. An example is the importance of altered metabolism. Some researchers believe that our entire way of thinking about cancer is fundamentally flawed, and that instead of viewing cancer as a genetic disease, we should be thinking of it as a metabolic disease.

Regardless, a number of new therapies have arisen from our more developed understanding of cancer biology. Increasing appreciation of the role of inflammation and the immune response has led to investigation of anti-inflammatory drugs and the development of vaccines. Currently, cancer vaccines are only effective in the prevention of viral-associated cancer. However, in 2010, the FDA approved the first therapeutic cancer vaccine, sipuleucel-T (Provenge, Dendreon Corporation) for prostate cancer.

Rational approaches to cancer management may also be found in therapies targeting energy metabolism. Preclinical studies are assessing the efficacy of small molecules targeting aerobic glycolysis, including 2-deoxyglucose, lonidamine, and 3-bromopyruvate, as well as glutamine-binding drugs, since glutamine is a major metabolite in many cancers.

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INPUT

Extracellular signals

Phosphorylation

Kinase 1

Kinase 2

Kinase 3

Secondary
phosphorylation

OUTPUT

Kinase 4
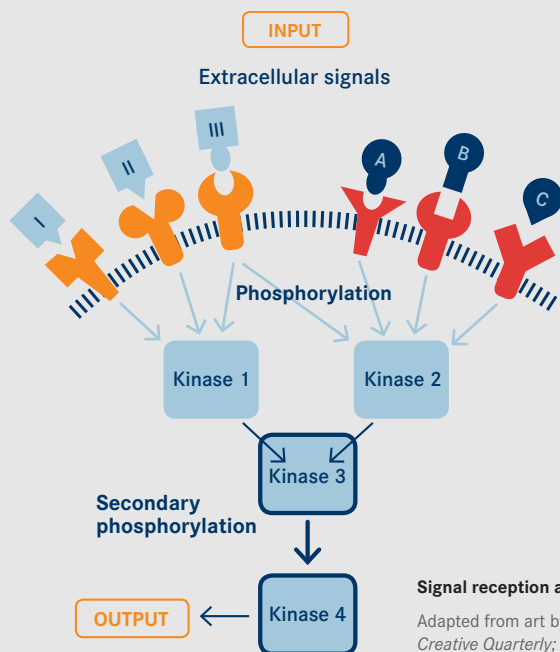
Signal reception activates cascade.
Adapted from art by Jane Wang, *The Science
Creative Quarterly*; August 2003.

The Science Behind The Treatment

A Look at the Role of Phosphorylation in Drug Design
and Patient Outcomes

By Jane de Lartigue, PhD

That phosphorylation is mentioned time and again in discussions of cell-signaling pathways in cancer is no coincidence. Since its discovery, phosphorylation has come to be recognized as a global regulator of cellular activity, playing a vital role in numerous intracellular processes, such as growth, proliferation, and cell division. Not surprisingly, aberrant phosphorylation is implicated in a host of human diseases. Phosphorylation is a vital and ubiquitous process that furthers our understanding and treatment of diseases such as cancer.

Phosphorylation Basics

Once a gene is expressed and translated into a functional cellular protein, the cell is able to control the protein's fate through the use of posttranslational modifications (PTMs). Phosphorylation is the most important and most thoroughly researched form of PTM. In essence, it can be viewed as an "on/off" switch for the particular protein being phosphorylated.

It's believed that as many as one-third of all proteins in the cell are phosphorylated at one time or another, and half of these proteins likely harbor more than 1 phosphorylation site, with different sites often eliciting quite different cellular responses.

Phosphorylation and the reverse reaction, dephosphorylation, occur via 2 key enzymes; protein kinases and phosphatases. Protein kinases phosphorylate proteins by transferring a phosphate group from adenosine triphosphate (ATP) to their target protein. This process is balanced by protein phosphatases, which can subsequently remove the phosphate group. In this way, the amount of phosphate associated with a protein is precisely determined by the relative activities of the associated kinase and phosphatase. As much as 2% to 5% of the human genome is thought to encode protein kinases and phosphatases. The most common amino acids to be phosphorylated on eukaryotic proteins (found in all organisms except bacteria) are serine, threonine, and tyrosine.

How It Works

At the level of a single protein, the binding of a negatively charged phosphate group can lead to changes in a protein's structure that alter the way it functions. If the targeted protein is an enzyme, phosphorylation and dephosphorylation can impact its enzymatic activity by essentially turning it on and off in a regulated manner. Another outcome of structural changes to the phosphorylated protein is the facilitation of binding to a partner protein; in this way, phosphorylation can regulate protein-protein interactions. Phosphorylation can also target a protein for degradation and removal from the cell via the ubiquitin-proteasome system.

Protein phosphorylation also has a vital role in intracellular signal transduction. Many of the proteins that make up a signaling pathway are kinases, from the tyrosine kinase receptors at the cell surface to downstream effector proteins, many of which are serine/threonine kinases. Ligand binding at the cell surface establishes a phosphorylation cascade, with the phosphorylation and activation of one protein stimulating the phosphorylation of another, subsequently amplifying a signal and transmitting it through the cell. The signal continues to propagate until being switched off by the action of a phosphatase.

In addition to proteins, other kinds of molecules can also be phosphorylated, in particular, phosphoinositide lipids.

The Cancer Connection

Because phosphorylation plays vital and varied roles in intracellular processes, any perturbations in the process are likely to drive many of the hallmarks of cancer, such as unchecked cell growth and proliferation. Mutations in kinases and phosphatases are often implicated in a number of different cancers, and many of the genes encoding for these proteins are oncogenes or tumor suppressors. According to Martin Steffen, MD, PhD, principal investigator at Boston University School of Medicine in the Departments of Pathology and Laboratory Medicine, and Biomedical Engineering, dysregulated protein phosphorylation can turn a protein "on" when, in normal tissue, the protein should be "off." This is especially true of signaling proteins that drive cellular proliferation. Overexpression or mutations that lead to constitutive activation of phosphorylation machinery disrupt the delicate balance in the cell, driving the inappropriate activation or deactivation of cellular processes.

Phosphorylation in Cancer Drug Development

The key function of protein kinases in signal transduction has made them an extremely attractive target for therapeutic intervention in cancer; protein kinases account for as much as 30% of all protein targets under investigation by pharmaceutical companies.

Targeting tyrosine kinases is a particularly popular approach, and groundbreaking advances have been made in recent decades with the introduction of this class of agents. More recently, the serine/threonine kinases have also emerged as strong candidates. Roughly one-third of all kinase inhibitors currently in development target serine/threonine kinases, with the most advanced of these agents aimed at the mammalian target of rapamycin (mTOR). A number of inhibitors targeting AKT are also in the pipeline, and there is substantial interest in drugs targeting MEK, a critical kinase at the junction of several biological pathways that regulate cell proliferation, survival, migration, and

> *Protein kinases account for as much as 30% of all protein targets under investigation by pharmaceutical companies.*

differentiation. Also undergoing intense investigation are the serine/threonine kinases involved in cell cycle regulation.

Biomarker Potential

Given the pivotal roles of phosphorylation in the cellular environment, and the fact that the overwhelming majority of phosphorylation sites remain uncharacterized, researchers are constantly striving to better understand the role of phosphorylation and to develop novel, highly sensitive, and sophisticated phosphorylation identification techniques.

The development of specific antibodies against phosphorylated forms of proteins has led to the use of immunoassays to examine phosphorylation patterns of individual proteins.

The potential for using the phosphorylation status of key signaling proteins as a diagnostic biomarker, predictor of response, or prognostic indicator is an area of intense interest. Investigators are examining the utility of phosphorylated forms of epidermal growth factor receptor and HER2 as both biomarkers of response to tyrosine kinase inhibitors and as prognostic indicators. For example, the findings from one study reveal that a high level of expression of phosphorylated HER2 in patients with HER2- positive breast cancer is associated with lower 5-year disease-free survival.

Another relatively new area of interest is the exploitation of binding domains within signaling proteins that specifically recognize and bind to other phosphorylated proteins. Using the Src homology 2 (SH2)-binding domain, which binds to phosphorylated tyrosines, researchers have been able to analyze the global state of tyrosine phosphorylation in different human cancer cell lines to see how it differs from normal cells.

"We believe that the patterns of phosphorylation in a cell will reveal the pathways that are activated in those tumors, and will therefore suggest which drugs might be efficacious for those tumors," said Steffen, who, partnering with Boston University's Simon Kasif, PhD, has already developed phosphorylation signatures that discriminate between lung tumors and normal lung, and is now developing signatures for the prediction of therapy response. Describing such endeavors as showing great promise, he noted that one ultimate goal would be "a comprehensive catalog of the phosphorylation status of all proteins in tumors," and another would be the ability to identify and comprehend a tumor's "weak points," with the aim of knowing which phosphorylated proteins could be targeted to maximally destroy the tumor cells while preserving normal tissue.

Jane de Lartigue, PhD, is a freelance medical writer and editor based in the United Kingdom.

Breast Cancer

Beyond Herceptin
How New HER2-Targeted Therapies Will Change the Way You Practice

The first drug that successfully targeted the HER2 pathway in cancer treatment, trastuzumab (Herceptin, Genentech), generated much excitement: In 2005, *The New England Journal of Medicine* heralded it as "a dramatic and perhaps permanent perturbation in the history of the disease, maybe even a cure." Since its approval in 1998, in fact, trastuzumab has become one of the most common therapeutic agents used in the treatment of breast cancer. Key to understanding the success of the drug is an understanding of the HER2 pathway itself.
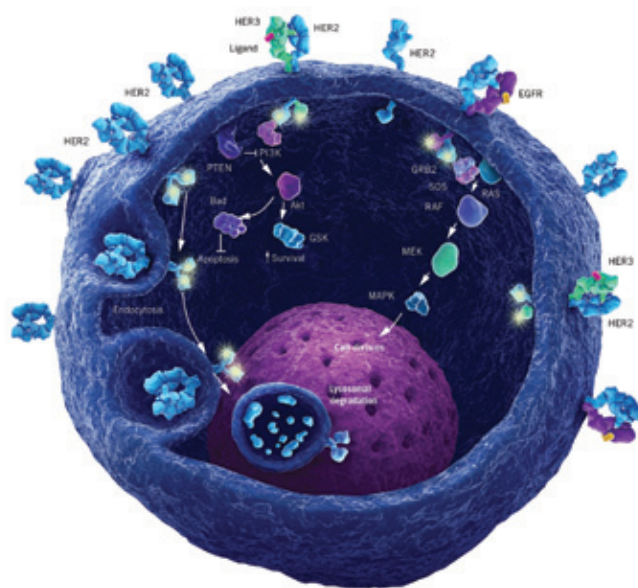
HER2 is a member of the human epidermal growth factor receptor (EGFR) family, along with 3 other proteins (HER1 or EGFR, HER3, and HER4). All are receptor tyrosine kinases that feed into a complex signaling network controlling numerous cellular processes, including proliferation, survival, differentiation, angiogenesis, invasion, and metastasis.

Dimerization is the process by which 2 receptors, such as HER2, join; it plays a key role in receptor signaling. Multiple ligands bind to the different HER receptors with different binding specificity, exposing an extracellular dimerization domain and enabling one receptor molecule to pair with another. The paired receptor molecules phosphorylate one another on tyrosine residues on their intracellular domains, enabling the activated receptors to bind to intracellular signaling molecules and initiate a variety of signaling pathways, including the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway.

HER2 has no known ligand, and therefore relies for activation on heterodimerization with other HER receptors, or homodimerization with itself when expressed at very high levels on the cell surface. The HER2 receptor remains in a constitutively active conformation. Consequently, HER2 is the preferred partner for other members of the HER family.

HER2 in Cancer Drug Development
HER2-containing heterodimers are the most prevalent and active of all the receptor pair combinations, making the HER2 pathway a target of significant interest for cancer researchers. Since the discovery of the

Members of the human epidermal growth factor receptor family.

Courtesy Genentech BioOncology, member of the Roche Group

HER2/neu oncogene in 1979, a high frequency and broad spectrum of aberrations in this pathway have been observed in human tumors. In 1984, Genentech researchers Axel Ullrich, PhD, and H. Michael Shepard, PhD, identified the protein produced by this gene and, together with Dennis Slamon, MD, PhD, discovered that the HER2 receptor is overexpressed in between 25% to 30% of breast cancers and is associated with more aggressive disease and a poorer outcome. More recent clinical data also support the role of HER2 in other tumor types, including gastric, thyroid, and head and neck cancers.

Trastuzumab, a monoclonal antibody, was the first HER2-targeted therapy to gain FDA approval for the treatment of HER2-positive breast cancer. Today, researchers are exploring its potential in combination therapy, sharpening the methods of identifying HER2 overexpression, and developing biomarkers to better identify potential candidates for treatment.

Despite the success of trastuzumab for early and advanced HER2-positive breast cancer—at least as a single, first-line agent—it did not prove to be the "cure" that many hoped it would be, with objective response rates of only 26%. Furthermore, many trastuzumab-treated patients experience relapse or progression, due mainly to the development of drug resistance via a number of possible mechanisms. As a result, efforts are under way to develop other HER2-targeted therapies to overcome these issues:

- Lapatinib (Tykerb, GlaxoSmithKline), a small-molecule tyrosine kinase inhibitor (TKI), and the only other HER2-targeted therapy currently on the market, was approved as a second-line therapy for HER2-positive disease in 2007, following reports that its addition to capecitabine (Xeloda, Genentech) improved patient survival over capecitabine alone. Because this dual-action TKI targets the intracellular domain of HER2 and HER1, it may be particularly useful in patients who lack p95, the extracellular domain to which HER2 binds.
- Pertuzumab (Roche), a humanized monoclonal antibody and the first in a new class of HER dimerization inhibitors, binds to a different

> *Up to 20% of current HER2 testing worldwide may deliver false positives or false negatives.*

epitope of the HER2 extracellular domain than trastuzumab and is able to block HER2 dimerization. It is currently undergoing phase III clinical trials for use in combination with trastuzumab **(see sidebar)**.

- Trastuzumab emtansine (T-DM1 [Roche Holding, AG]), an antibody-drug conjugate currently in development, is a combination of trastuzumab conjugated to a potent cytotoxic agent (mytansine derivative [ImmunoGen, Inc]) by a stable thioether linker. Clinical trials have demonstrated that trastuzumab-resistant patients are sensitive to this drug, with response rates of approximately 30%.

Other HER2-targeted therapies in development include AEE788 (Novartis Pharmaceuticals), a multitargeted HER1/2 and vascular endothelial growth factor receptor inhibitor; afatinib (Boehringer Ingelheim), an EGFR/HER2 inhibitor; and canertinib (Pfizer Inc), a pan-HER TKI. Additionally, the HER2 TKIs CP-724, 714 (Axon Medchem BV), and neratinib (Pfizer Inc), and TAK-285 (Millennium/Takeda Pharmaceutical Company Ltd), a dual HER2/EGFR TKI, are currently in development.

The kinetic pace of trastuzumab development highlights 2 significant findings already shaping the future of targeted therapies: (1) single agents are not sufficient to completely target complex signaling networks, and (2) it is vital to identify patients (via molecular testing) who will most likely respond to targeted therapies.

There are currently several FDA-approved methods for evaluating HER2 expression: fluorescence in situ hybridization (FISH) and immunohistochemistry via HerceptTest™ (Dako), or the HER2/neu rabbit monoclonal antibody test (Ventana Medical Systems, Inc). Considerable controversy exists over defining HER2 overexpression. Up to 20% of current HER2 testing worldwide may deliver false positives or false negatives, according to the American Society of Clinical Oncology (ASCO)—an issue being examined through the North Central Cancer Treatment Group N9831 Adjuvant Trastuzumab Trial. Finally, the FDA recently approved a new test (Inform Dual ISH) for detecting HER2 overexpression.

HER2 and Dual Inhibition

Several new studies suggest that dual inhibition of HER2 might serve as a better approach to treating patients with HER2-positive breast cancer.

Dramatic findings in the CLEOPATRA (CLinical Evaluation Of Pertuzumab And TRAstuzumab) trial showed that combining pertuzumab with trastuzumab (Herceptin) and docetaxel chemotherapy reduced the risk of progression by 38% in women with metastatic HER2-positive disease. Trastuzumab, a monoclonal antibody, targets HER2, while pertuzumab is the first in a new class of HER2 dimerization inhibitors that prevents HER2 receptor proteins from linking to other HER receptors, according to Genentech, which is developing the drug. The findings likely will result in a "sea change" in the way the disease is treated, said principal investigator José Baselga, MD, PhD, a professor in the Department of Medicine at Harvard Medical School and chief of Hematology/Oncology at Massachusetts General Hospital in Boston.

CLEOPATRA enrolled 808 women with confirmed HER2-positive, locally recurrent, unresectable, or metastatic disease. At median follow-up of 19.3 months, patients who received pertuzumab (n = 402) had a median progression-free survival (PFS) of 18.5 months. By comparison, patients who received trastuzumab, docetaxel, and a placebo (n = 406) demonstrated a median PFS of 12.4 months (hazard ratio = 0.62; confidence interval, 0.51-0.75; P = <.0001). The objective response rate (tumor shrinkage ≥30%) was 80.2% in the pertuzumab group versus 69.3% in the placebo arm.

As a result of the positive findings, Genentech has submitted an application for pertuzumab to be used for patients with previously untreated HER2-positive metastatic breast cancer, and the combined regimen is now under study in patients with early-stage disease.

Another study, Neo ALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization), published online in *The Lancet*, combined lapatinib (Tykerb, a tyrosine kinase inhibitor) and trastuzumab. The randomized, open-label, phase III study assigned patients to receive lapatinib (n =154), trastuzumab (n =149), or a combination of the 2 therapies (n =152). The primary endpoint of the trial was the rate of pathological complete response (pCR) at the time of surgery.

Patients in the combination arm had a significantly higher rate of pCR (51.3%; 95% CI, 43.1%-59.5%) than patients who received trastuzumab alone (29.5%; 95% CI, 22.4%-37.5%) or lapatinib alone (24.7%; 95% CI, 18.1%-32.3%).

"This is the first demonstration that adding a second anti-HER2 therapy, lapatinib, to trastuzumab is superior to trastuzumab alone in patients with early breast cancer," said Baselga, lead author of the study. "It opens up the concept of dual HER2 blockade as a better approach for patients with early, nonmetastatic, HER2 breast cancer."

In another study, in *The Lancet Oncology*, trastuzumab was compared to lapatinib when each was combined with neoadjuvant anthracycline-taxane-based chemotherapy (GeparQuinto, GBG 44). The randomized phase III trial enrolled 620 eligible patients with untreated HER2-positive, operable, or locally advanced breast cancer: 309 received chemotherapy with trastuzumab, while the remaining 311 patients received chemotherapy with lapatinib.

A total of 30.3% of patients in the trastuzumab arm (n = 93) and 22.7% of patients in the lapatinib arm (n = 70) achieved pCR (OR = 0.68; 95% CI, 0.47-0.97; P = .04). Because the rate of pCR was lower in the lapatinib arm, the authors suggest that the drug should not be administered as a single-agent anti-HER2 treatment outside of clinical trial.

In a commentary accompanying the study, Stephen K. Chia, MD, a senior scientist in the Division of Medical Oncology at the British Columbia Cancer Agency, wrote that "Moving forward into the future, no adjuvant trials should be done without an adequate signal from preoperative trials showing safety, efficacy, target modulation, and, ideally, the identification of predictive biomarkers such that we no longer pick a loser to study in larger and more resource-intensive adjuvant trials."

Are You Ready for PI3K/Akt Inhibitors?
After 20 Years of Research, These New Therapies Are Poised to Enter the Market

In 2011, more than 20 years of research into the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway was marked with numerous conferences focusing on this rapidly growing area of research. This focus is well deserved, since PI3K/Akt is one of the most perturbed signaling pathways in human cancer, and is an integral cog in a network of other high-profile signaling pathways.
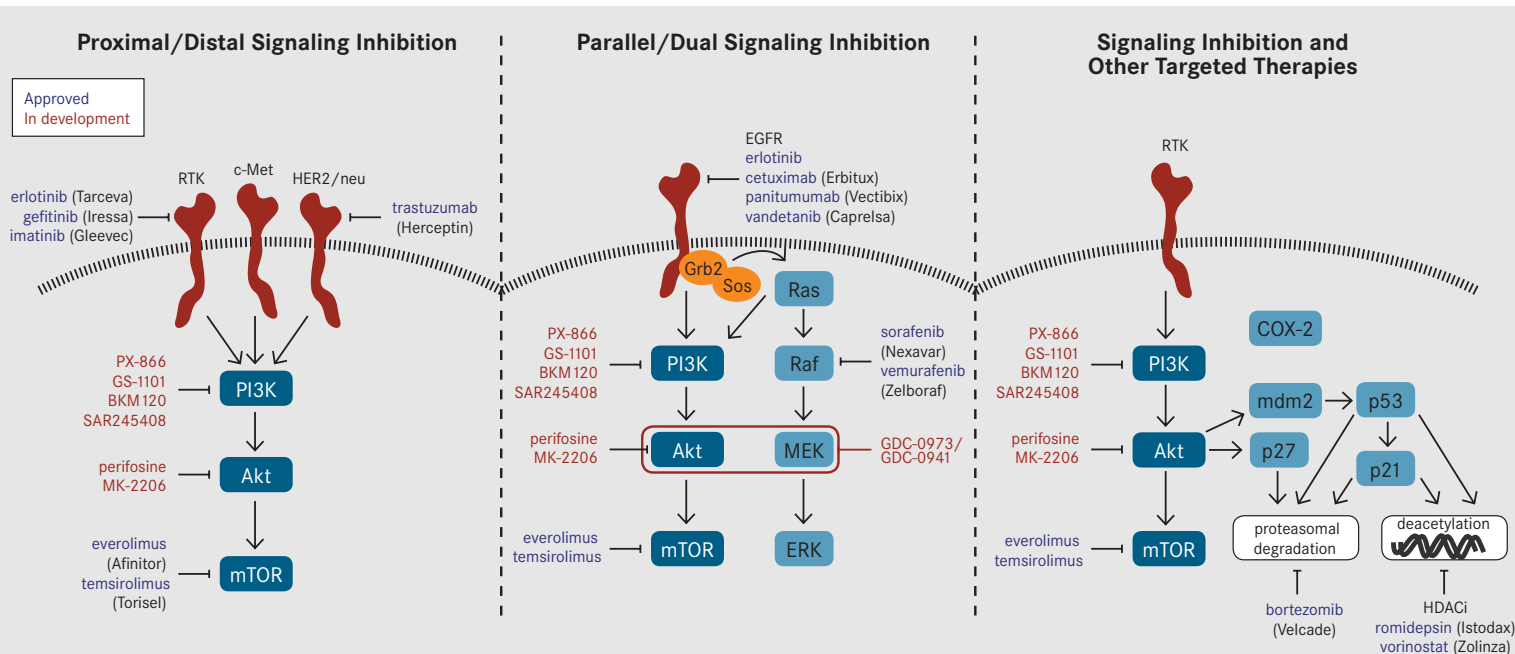
The PI3Ks are a large family typically divided into 3 classes. The majority of research has focused on the class I PI3Ks, which have the best characterized role in human cancer. Class I PI3Ks are further subdivided into classes IA and IB, and are activated at the cell surface by 2 types of membrane receptors: receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), and G protein-coupled receptors.

The activation of Akt stimulates a variety of critical physiological cellular metabolic and survival functions. In normal cells, the primary biochemical function of the PI3Ks is to phosphorylate the 3-hydroxyl group of phosphoinositides, which results in activation of second messengers, the most important one being the serine/threonine kinase Akt. Because the best studied target of PI3K/Akt signaling is the mammalian target of rapamycin (mTOR), it is frequently referred to as the PI3K/Akt/mTOR pathway.

~ Jane de Lartigue, PhD

Proximal/Distal Signaling Inhibition

Approved
In development

erlotinib (Tarceva)
gefitinib (Iressa)
imatinib (Gleevec)

RTK · c-Met · HER2/neu

trastuzumab (Herceptin)

PX-866
GS-1101
BKM120
SAR245408 — PI3K

perifosine
MK-2206 — Akt

everolimus (Afinitor)
temsirolimus (Torisel) — mTOR

Parallel/Dual Signaling Inhibition

EGFR
erlotinib
cetuximab (Erbitux)
panitumumab (Vectibix)
vandetanib (Caprelsa)

Grb2 · Sos · Ras

PX-866
GS-1101
BKM120
SAR245408 — PI3K · Raf — sorafenib (Nexavar) vemurafenib (Zelboraf)

perifosine
MK-2206 — Akt · MEK — GDC-0973/GDC-0941

everolimus
temsirolimus — mTOR · ERK

Signaling Inhibition and Other Targeted Therapies

RTK

PX-866
GS-1101
BKM120
SAR245408 — PI3K

COX-2

perifosine
MK-2206 — Akt — mdm2 → p53

p27

p21

everolimus
temsirolimus — mTOR

proteasomal degradation — bortezomib (Velcade)

deacetylation — HDACi romidepsin (Istodax) vorinostat (Zolinza)

Approaches under study related to the P13K/Akt pathway

The 3 panels illustrate ways in which FDA-approved and investigational agents may be combined. Agents aimed at receptor tyrosine kinases (RTKs) and oncogenes may be combined with agents that inhibit the pathway at key junctures (left). Dual inhibition of parallel signaling pathways is another option (center). Agents also may be combined with histone deacetylase complex inhibition (HDACi) and other targeted therapies (right).

Adapted from LoPiccolo J, Blumenthal GM, Bernstein WB, Dennis PA. Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. *Drug Resist Updat.* 2008;11(1-2):32-50.

PI3K in Cancer Drug Discovery

Because of its crucial role in many aspects of cell growth and survival, PI3K/Akt is almost invariably found "switched on" in a myriad of cancers, and also has prognostic and predictive relevance in many human tumors. In fact, with the exception of the p53 tumor suppressor pathway, the PI3K pathway is the most highly perturbed in human cancer. Common alterations include mutations in the PIK3CA gene, encoding the p110 catalytic subunit, which are observed in more than 50% of bowel cancers and 26% of all breast cancers, and loss of expression of PTEN, observed in more than 50% of all cancers, including glioma, melanoma, and prostate cancer. AKT amplification, mutation, and over-expression are found in head and neck, gastric, ovarian, pancreatic, and colorectal cancers (CRC).

Described as "one of the most exciting targets for cancer therapeutics," PI3K/Akt has been a major focus of research and cancer drug development by a multitude of pharmaceutical companies and academic centers.

A host of small-molecule inhibitors directly targeting PI3K, Akt, and other key nodes in this pathway are at various stages of clinical development. The discovery of oncogenic mutations in the PIK3CA gene and elucidation of crystal structures of the catalytic domain of different PI3K isoforms have aided in the development of more isoform-specific PI3K inhibitors. These include GS-1101 (Gilead Sciences, Inc), targeting the p110 delta isoform, which is in phase II clinical trials for chronic lymphocytic leukemia and non-Hodgkin lymphoma, and PX-866 (Oncothyreon), targeting the p110 alpha isoform, in phase II trials for brain cancer. Inhibitors targeting multiple isoforms of the class I PI3Ks (known as pan-PI3K inhibitors) are also in development, including BKM120 (Novartis) in phase II trials for uterine cancer and non-small cell lung cancer (NSCLC) and SAR245408 (sanofi -aventis), in phase II trials for uterine and breast cancer.

Specific Akt inhibitors are also in the pipeline, particularly perifosine (KRX-0401, Keryx Biopharmaceuticals, Inc), currently in phase III trials for multiple myeloma and CRC, with results due early this year. Another Akt inhibitor, MK-2206 (Merck & Co, Inc), is undergoing phase I clinical testing in advanced solid tumors. Thus far, there has been limited clinical response to these drugs as monotherapy, even among patients with PIK3CA and PTEN alterations, and evidence suggests that these drugs may have the most clinical benefit when used in combination— either with other PI3K/Akt inhibitors, with other targeted therapies, or with conventional treatments such as chemotherapy—and this has subsequently become an area of intensive research.

There is also substantial interest in the combination of PI3K/Akt agents with inhibitors of the mitogen-activated protein kinase (MAPK) pathway. A phase IB trial of Roche's GDC-0973 (a MEK inhibitor) and GDC-0941 (a PI3K inhibitor) is under way and has shown signs of antitumor activity in patients with advanced solid tumors.

Furthermore, because extensive crosstalk exists between PI3K/Akt and other important signaling pathways, the inhibition of mTOR inadvertently leads to feedback activation of Akt. For this reason, combining PI3K/Akt and mTOR targeted agents may be particularly beneficial. In fact, dual PI3K/mTOR inhibitors are also under development, including BEZ235 (Novartis) and SAR245409 (sanofi-aventis), in phase II clinical trials for uterine and breast cancer, respectively.

Yet another area of interest is PI3K/Akt's apparent role in treatment resistance. Experience with targeted agents against mTOR and RTKs has demonstrated that cancer cells are highly adept at keeping the PI3K/Akt pathway switched on despite inhibitor activity, and residual PI3K/Akt pathway activation has frequently been described in cells that have developed resistance to these therapies. For example, PI3K has been shown to play a key role in resistance to HER2-directed agents such as the monoclonal antibody trastuzumab and tyrosine kinase inhibitor (TKI) lapatinib, and combination with PI3K/Akt-targeted agents has been observed to restore their therapeutic effects.
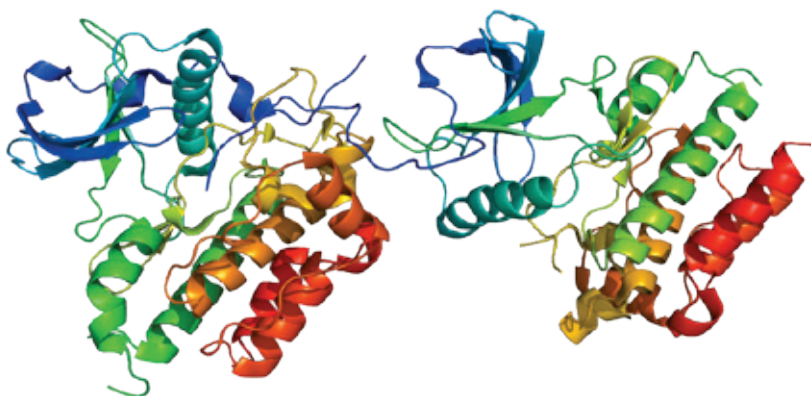
GIST

Structure of the KIT protein

GIST, Melanoma, and More
A Better Understanding of the SCF/KIT Pathway Will Allow for More Precisely Targeted Therapy

The importance of the stem cell factor (SCF)/KIT signaling pathway in normal human physiology is relatively well understood, but the breadth of its oncogenic functions and potential role as a therapeutic target are only now becoming clear.

The SCF/KIT pathway is believed to have a number of major in vivo functions in humans. Like the JAK-STAT pathway **(see page 28)**, SCF/KIT signaling is crucial for hematopoiesis. It plays a vital role in the development of primitive hematopoietic cells such as stem and progenitor cells.

SCF/KIT also has a well-established role in melanocyte development, with KIT mutations detected in the majority of individuals with a rare inherited condition called piebaldism. SCF/KIT signaling is also important in the gastrointestinal tract (specifically in the development of the interstitial cells of Cajal), in fertility, and in the central nervous system.

The significant genetic player in this pathway is the *KIT* gene, whose KIT protein product is a member of the class III receptor tyrosine kinases (RTKs), a family that includes other receptors such as platelet-derived growth factor receptor. As with other RTKs, the binding of a specific ligand, in this case SCF, leads to receptor dimerization, which activates the receptor's intrinsic tyrosine kinase activity, leading to its phosphorylation at key amino acid residues. These residues subsequently act as binding sites for

signaling molecules within the cell; signal transduction is initiated downstream of the receptor. A number of downstream signaling cascades are activated by the SCF/KIT pathway, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling.

SCF/KIT in Cancer Drug Development

KIT is associated with several human malignancies, including melanoma, mastocytosis, gastrointestinal stromal tumors (GISTs), acute myelogenous lymphoma, and small cell lung carcinoma (SCLC). KIT also has been described in a range of other tumors, such as neuroblastoma, cervical, testicular, thyroid, breast, colon, bladder, renal, pancreatic, and non-small cell lung carcinoma (NSCLC), but its precise role is often complex and poorly understood.

KIT is overexpressed in some melanomas, but progression to invasive and metastatic stages has also been shown to correlate with reduced levels of the protein, suggesting a tumor-suppressive function. In the majority of cases, however, tumors arise from activating mutations in *KIT* or its overexpression; for example, 50% to 80% of GISTs have an activating mutation in *KIT*, and 28% to 88% of SCLC cell lines have been reported to overexpress KIT. Furthermore, mutations in the kinase domain (D816V) of *KIT* have been identified in the mast cells of patients with mastocytosis.

The Road Back to Imatinib

Although no KIT-specific inhibitors have been generated to date, imatinib is multitargeted, and also inhibits KIT activity. When it comes to tumors associated with KIT abnormalities, GISTs have been the real success story for imatinib. Prior to the 2001 approval of the so-called "magic bullet," patients with GISTs that harbor a *KIT* mutation had a particularly poor prognosis. Imatinib revolutionized the treatment of these tumors, with more than 80% of patients responding.

Not all *KIT*-defective tumors have displayed such dramatic effects; imatinib proved relatively ineffective for mastocytosis and controversial in the treatment of melanomas, with several phase II studies reporting no clinical activity in metastatic melanoma. Furthermore, imatinib-treated GIST patients developed drug resistance, and researchers began to look for alternatives. Sunitinib (Sutent, Pfizer) was the first targeted therapy to show effectiveness in treating imatinib-refractory GISTs. Subsequently, clinical trial data presented at the 2011 ASCO meeting highlighted a number of second-generation TKIs that include KIT among their targets and demonstrate significant activity in imatinib-resistant GISTs, including sorafenib (Nexavar, Bayer HealthCare and Onyx Pharmaceuticals), nilotinib (Tasigna, Novartis), dasatinib (Sprycel, Bristol-Myers Squibb), and regorafenib (BAY 73-4506, Bayer HealthCare).

Today, the molecular analysis of tumors has led us back to imatinib. We now understand the importance of screening patients for mutations in *KIT* known to play a role in melanoma development, and the case is slowly being made for reassessment of this agent for its treatment. Substantial evidence now suggests that melanoma cell lines with *KIT* mutations are sensitive to imatinib, and that patients with such mutations can experience significant and durable clinical responses.

~ Jane de Lartigue, PhD

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When PTCL Returns…

BE READ

WITH FOLO

(pralatrexate

FOLOTYN is indicated for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma. The indication for FOLOTYN is based on overall response rate. Clinical benefit such as improvement in progression-free survival or overall survival has not been demonstrated.

Demonstrated response in relapsed or refractory PTCL[1]

27% overall response rate (CR+CRu+PR)
by independent central review (95% CI, 19-36)*

Of the responders,

66% responded within Cycle 1*
—Median time to first response was 45 days (range=37-349 days)

9.4-month median duration of response by central review (range=1-503 days)*
—12% (95% CI, 7-20) of patients had responses lasting ≥14 weeks (range=98-503 days)

Demonstrated response in

PROPEL—
the first large, prospective, single-arm, open-label clinical trial in PTCL

Adverse Reactions
The most common adverse reactions were mucositis (70%), thrombocytopenia (41%), nausea (40%), and fatigue (36%). The most common serious adverse events were pyrexia, mucositis, sepsis, febrile neutropenia, dehydration, dyspnea, and thrombocytopenia.

Use in Specific Patient Populations
Nursing mothers should be advised to discontinue nursing or the drug, taking into consideration the importance of the drug to the mother.

Drug Interactions
Co-administration of drugs subject to renal clearance (e.g., probenecid, NSAIDs, and trimethoprim/ sulfamethoxazole) may result in delayed renal clearance.

Please see FOLOTYN Full Prescribing Information.

*Per independent central review

Reference: 1. FOLOTYN Prescribing Information. Allos Therapeutics, Inc., 2011.

FOLOTYN
(pralatrexate injection)

summary of Prescribing Information.

www.FOLOTYN.com

Brief summary of Full Prescribing Information for FOLOTYN® (pralatrexate injection)—Please consult Full Prescribing Information.

INDICATIONS AND USAGE

FOLOTYN is indicated for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL). This indication is based on overall response rate. Clinical benefit such as improvement in progression-free survival or overall survival has not been demonstrated.

WARNINGS AND PRECAUTIONS

Bone Marrow Suppression
FOLOTYN can suppress bone marrow function, manifested by thrombocytopenia, neutropenia, and anemia. Dose modifications are based on ANC and platelet count prior to each dose.

Mucositis
Treatment with FOLOTYN may cause mucositis. If ≥Grade 2 mucositis is observed, omit dose and follow guidelines in Table 1.

Dermatologic Reactions
FOLOTYN has been associated with severe dermatologic reactions, which may result in death. These dermatologic reactions have been reported in clinical studies (14/663 patients [2.1%]) and post marketing experience, and have included skin exfoliation, ulceration, and toxic epidermal necrolysis (TEN). These reactions may be progressive and increase in severity with further treatment, and may involve skin and subcutaneous sites of known lymphoma. Patients with dermatologic reactions should be monitored closely, and if severe, FOLOTYN should be withheld or discontinued.

Tumor Lysis Syndrome
Tumor lysis syndrome has been reported in patients with lymphoma receiving FOLOTYN. Patients receiving FOLOTYN should be monitored closely and treated for complications.

Folic Acid and Vitamin B₁₂ Supplementation
Patients should be instructed to take folic acid and receive vitamin B₁₂ to potentially reduce treatment-related hematological toxicity and mucositis.

Pregnancy Category D
FOLOTYN can cause fetal harm when administered to a pregnant woman. FOLOTYN was embryotoxic and fetotoxic in rats and rabbits. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Decreased Renal Function
Although FOLOTYN has not been formally tested in patients with renal impairment, caution is advised when administering FOLOTYN to patients with moderate to severe impairment. Monitor patients for renal function and systemic toxicity due to increased drug exposure.

Elevated Liver Enzymes
Liver function test abnormalities have been observed after FOLOTYN administration. Persistent liver function test abnormalities may be indicators of liver toxicity and require dose modification. Monitor patients for liver function.

ADVERSE REACTIONS
The most common adverse reactions observed in patients with peripheral t-cell lymphoma (PTCL) treated with FOLOTYN were mucositis, thrombocytopenia, nausea, and fatigue.

Clinical Trials Experience
Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of a drug cannot be directly compared to rates in the clinical studies of another drug and may not reflect the rates observed in practice.

The safety of FOLOTYN was evaluated in 111 PTCL patients in a single-arm clinical study in which patients received a starting dose of 30 mg/m² once weekly for 6 weeks in 7-week cycles. The median duration of treatment was 70 days (range 1-540 days).

Most Frequent Adverse Reactions
Table 4 summarizes the most frequent adverse reactions, regardless of causality, using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI CTCAE, version 3.0).

Table 4 Adverse Reactions Occurring in PTCL Patients (Incidence ≥10% of patients)

Preferred Term	N=111 Total N	Total %	Grade 3 N	Grade 3 %	Grade 4 N	Grade 4 %
Any Adverse Event	111	100	48	43	34	31
Mucositis[a]	78	70	19	17	4	4
Thrombocytopenia[b]	45	41	15	14	21	19[b]
Nausea	44	40	4	4	0	0
Fatigue	40	36	5	5	2	2
Anemia	38	34	17	15	2	2
Constipation	37	33	0	0	0	0
Pyrexia	36	32	1	1	1	1
Edema	33	30	1	1	0	0
Cough	31	28	1	1	0	0
Epistaxis	29	26	0	0	0	0
Vomiting	28	25	2	2	0	0
Neutropenia	27	24	14	13	8	7
Diarrhea	23	21	2	2	0	0
Dyspnea	21	19	8	7	0	0
Anorexia	17	15	3	3	0	0

Preferred Term	N=111 Total N	Total %	Grade 3 N	Grade 3 %	Grade 4 N	Grade 4 %
Hypokalemia	17	15	4	4	1	1
Rash	17	15	0	0	0	0
Pruritus	16	14	2	2	0	0
Pharyngolaryngeal pain	15	14	1	1	0	0
Liver function test abnormal[c]	14	13	6	5	0	0
Abdominal pain	13	12	4	4	0	0
Pain in extremity	13	12	0	0	0	0
Back pain	12	11	3	3	0	0
Leukopenia	12	11	3	3	4	4
Night sweats	12	11	0	0	0	0
Asthenia	11	10	1	1	0	0
Tachycardia	11	10	0	0	0	0
Upper respiratory tract infection	11	10	1	1	0	0

[a] Stomatitis or mucosal inflammation of the gastrointestinal and genitourinary tracts
[b] Five patients with platelets <10,000/μL
[c] Alanine aminotransferase, aspartate aminotransferase, and transaminases increased

Serious Adverse Events
Forty-four percent of patients (n=49) experienced a serious adverse event while on study or within 30 days after their last dose of FOLOTYN. The most common serious adverse events (>3%), regardless of causality, were pyrexia, mucositis, sepsis, febrile neutropenia, dehydration, dyspnea, and thrombocytopenia. One death from cardiopulmonary arrest in a patient with mucositis and febrile neutropenia was reported in this trial. Deaths from mucositis, febrile neutropenia, sepsis, and pancytopenia occurred in 1.2% of patients on all FOLOTYN trials at doses ranging from 30 to 325 mg/m².

Discontinuations
Twenty-three percent of patients (n=25) discontinued treatment with FOLOTYN due to adverse reactions. The adverse reactions reported most frequently as the reason for discontinuation of treatment were mucositis (6%, n=7) and thrombocytopenia (5%, n=5).

Dose Modifications
The target dose of FOLOTYN was 30 mg/m² once weekly for 6 weeks in 7-week cycles. The majority of patients (69%, n=77) remained at the target dose for the duration of treatment. Overall, 85% of scheduled doses were administered.

Post Marketing Experience
Toxic epidermal necrolysis has been identified during post approval use of FOLOTYN. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure (see Warnings and Precautions).

DRUG INTERACTIONS
In vitro studies indicate that pralatrexate is not a substrate, inhibitor, or inducer of CYP450 isoenzymes and has low potential for drug-drug interactions at CYP450 isoenzymes. No formal clinical assessments of pharmacokinetic drug-drug interactions between FOLOTYN and other drugs have been conducted. The effect of co-administration of the uricosuric drug probenecid on pralatrexate pharmacokinetics was investigated in a Phase 1 clinical study. Co-administration of increasing doses of probenecid resulted in delayed clearance of pralatrexate and a commensurate increase in exposure.

Due to the contribution of renal excretion (approximately 34%) to the overall clearance of pralatrexate, concomitant administration of drugs that are subject to substantial renal clearance (eg, NSAIDs, trimethoprim/sulfamethoxazole) may result in delayed clearance of pralatrexate.

USE IN SPECIFIC POPULATIONS

Pregnancy
Pregnancy Category D (see Warnings and Precautions).
FOLOTYN can cause fetal harm when administered to a pregnant woman. Pralatrexate was embryotoxic and fetotoxic in rats at IV doses of 0.06 mg/kg/day (0.36 mg/m²/day or about 1.2% of the clinical dose on a mg/m² basis) given on gestation days 7 through 20. Treatment with pralatrexate caused a dose-dependent decrease in fetal viability manifested as an increase in late, early, and total resorptions. There was also a dose-dependent increase in post-implantation loss. In rabbits, IV doses of 0.03 mg/kg/day (0.36 mg/m²/day) or greater given on gestation days 8 through 21 also caused abortion and fetal lethality. This toxicity manifested as early and total resorptions, post-implantation loss, and a decrease in the total number of live fetuses. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Nursing Mothers
It is not known whether pralatrexate is excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from this drug, a decision should be made whether to discontinue nursing or to discontinue FOLOTYN, taking into account the importance of FOLOTYN to the mother.

Pediatric Use
Pediatric patients were not included in clinical studies with FOLOTYN. The safety and effectiveness of FOLOTYN in pediatric patients have not been established.

Geriatric Use
In the PTCL efficacy study, 36% of patients (n=40) were 65 years of age and over. No overall differences in efficacy and safety were observed in patients based on age (<65 years compared with ≥65 years).

No dosage adjustment is required in elderly patients with normal renal function.

Hepatic Impairment
Formal studies have not been performed with FOLOTYN in patients with hepatic impairment. Patients with the following laboratory values were excluded from the pralatrexate lymphoma clinical trials: total bilirubin >1.5 mg/dL; aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 × upper limit of normal (ULN); and AST or ALT >5 × ULN if documented hepatic involvement with lymphoma.

Renal Impairment
See Warnings and Precautions.

OVERDOSAGE
No specific information is available on the treatment of overdosage of FOLOTYN. If an overdose occurs, general supportive measures should be instituted as deemed necessary by the treating physician. Based on FOLOTYN'S mechanism of action the prompt administration of leucovorin should be considered.

PATIENT COUNSELING INFORMATION
See FDA-approved Patient Package Insert.
Patients should be instructed to read the Patient Package Insert carefully.

DOSAGE AND ADMINISTRATION
FOLOTYN should be administered under the supervision of a qualified physician experienced in the use of antineoplastic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available.

Peripheral T-cell Lymphoma
The recommended dose of FOLOTYN is 30 mg/m² administered as an intravenous (IV) push over 3-5 minutes via the side port of a free-flowing 0.9% Sodium Chloride Injection, USP IV line once weekly for 6 weeks in 7-week cycles until progressive disease or unacceptable toxicity.

Vitamin Supplementation
Patients should take low-dose (1.0-1.25 mg) oral folic acid on a daily basis. Folic acid should be initiated during the 10-day period preceding the first dose of FOLOTYN, and dosing should continue during the full course of therapy and for 30 days after the last dose of FOLOTYN. Patients should also receive a vitamin B₁₂ (1 mg) intramuscular injection no more than 10 weeks prior to the first dose of FOLOTYN and every 8-10 weeks thereafter. Subsequent vitamin B₁₂ injections may be given the same day as treatment with FOLOTYN (see Warnings and Precautions).

Monitoring and Dose Modifications
Management of severe or intolerable adverse reactions may require dose omission, reduction, or interruption of FOLOTYN therapy.

Monitoring
Complete blood cell counts and severity of mucositis should be monitored weekly. Serum chemistry tests, including renal and hepatic function, should be performed prior to the start of the first and fourth dose of a given cycle.

Dose Modification Recommendations
Prior to administering any dose of FOLOTYN:
- Mucositis should be ≤Grade 1.
- Platelet count should be ≥100,000/μL for first dose and ≥50,000/μL for all subsequent doses.
- Absolute neutrophil count (ANC) should be ≥1,000/μL.

Doses may be omitted or reduced based on patient tolerance. Omitted doses will not be made up at the end of the cycle; once a dose reduction occurs for toxicity, do not re-escalate. For dose modifications and omissions, use the guidelines in Tables 1, 2, and 3.

Table 1 FOLOTYN Dose Modifications for Mucositis

Mucositis Grade[a] on Day of Treatment	Action	Dose upon Recovery to ≤Grade 1
Grade 2	Omit dose	Continue prior dose
Grade 2 recurrence	Omit dose	20 mg/m²
Grade 3	Omit dose	20 mg/m²
Grade 4	Stop therapy	

[a] Per National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI CTCAE, Version 3.0)

Table 2 FOLOTYN Dose Modifications for Hematologic Toxicities

Blood Count on Day of Treatment	Duration of Toxicity	Action	Dose upon Restart
Platelet <50,000/μL	1 week	Omit dose	Continue prior dose
	2 weeks	Omit dose	20 mg/m²
	3 weeks	Stop therapy	
ANC 500-1,000/μL and no fever	1 week	Omit dose	Continue prior dose
ANC 500-1,000/μL with fever or ANC <500/μL	1 week	Omit dose, give G-CSF or GM-CSF support	Continue prior dose with G-CSF or GM-CSF support
	2 weeks or recurrence	Omit dose, give G-CSF or GM-CSF support	20 mg/m² with G-CSF or GM-CSF support
	3 weeks or 2nd recurrence	Stop therapy	

Table 3 FOLOTYN Dose Modifications for All Other Treatment-related Toxicities

Toxicity Grade[a] on Day of Treatment	Action	Dose upon Recovery to ≤Grade 2
Grade 3	Omit dose	20 mg/m²
Grade 4	Stop therapy	

[a] Per National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI CTCAE, Version 3.0)

ALLOS® THERAPEUTICS

When Targeted Therapies Don't Work

What Are Your Options for Overcoming Resistance to Drugs That Target EGFR?
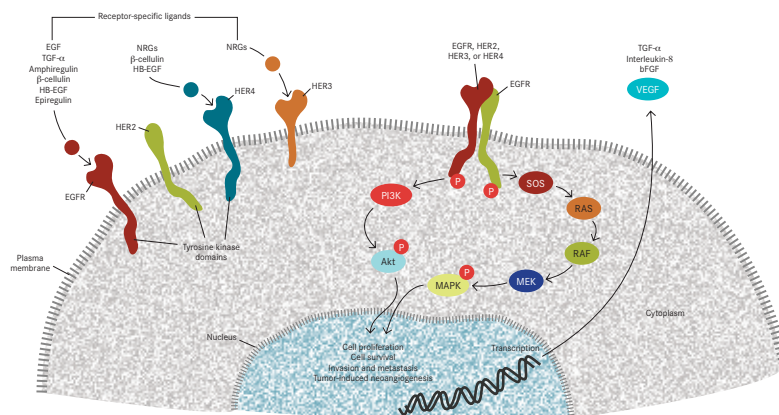
One of the most comprehensively studied molecular targets in clinical oncology, the epidermal growth factor receptor (EGFR), was the first receptor tyrosine kinase (RTK) to be identified. Dysregulation of the EGFR pathway by overexpression or constitutive activation can promote 5 of the 6 hallmarks of cancer. Defects in the EGFR pathway have been implicated in a diverse range of cancers, including colorectal, head and neck, lung, breast, and pancreatic.

EGF denotes epidermal growth factor; EGFR, epidermal growth factor receptor; HB, heparin-binding; HER, human epidermal growth factor receptor; NRG, neuregulin; bFGF, basic fibroblast growth factor; P, phosphate; TGF, transforming growth factor. Adapted from Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med*. 2008;358:1160-1174.

The Resistance Challenge

Resistance, both intrinsic and acquired, has posed significant challenges to the optimal use of EGFR inhibitors.

One challenge: Some mutations in the *EGFR* gene are activating and drive tumor formation, but others could actually be governing intrinsic resistance against EGFR inhibitors. Intrinsic resistance is also reported to be conferred by the presence of mutations in the *KRAS* gene.

In patients with non-small cell lung cancer (NSCLC), acquired resistance to TKIs occurs after a median of 10 to 14 months of treatment. Several mechanisms of acquired resistance have been reported, the most common of which is T790M, a gatekeeper mutation that restores ATP binding and permits tyrosine kinase activity even in the presence of inhibitors. In more than 50% of cases, T790M is responsible for TKI resistance. In response to this mutation, a number of novel agents are being developed, including irreversible inhibitors and EGFR mutation–specific antibodies. Another major mechanism of acquired resistance is compensatory amplification of the *MET* gene. MET is able to activate an alternate member of the ERBB family, ERBB3, and sustain activation of the PI3K/Akt cascade.

Other theories for acquired resistance to EGFR inhibitors include ubiquitination, which targets EGFR for destruction in the cell; the epithelial-to-mesenchymal transition, in recognition of the fact that mesenchymal cells are more resistant; oncogenic shift, involving increased expression of alternative ERBB receptors; and activation of alternative signaling pathways. As a result, EGFR inhibitors are also being tested in combination with other agents, including vascular endothelial growth factor receptor and MET TKIs.

The Biomarker Conundrum

Another challenge is the urgent need for improved methods of selecting optimal candidates. Phase III trials of these agents have demonstrated substantial variability, with some demonstrating no improvement in overall survival or time to progression. Crucially, the Iressa Pan-Asia Study found that gefitinib had only about a 1% response rate in patients with NSCLC who did not have activating mutations of EGFR, compared with a nearly 25% response rate for chemotherapy, demonstrating no benefit from gefitinib.

Furthermore, though protein overexpression has proved to be a useful biomarker of response to targeted therapies for other oncogenic pathways, most studies have failed to show any significant relationship between EGFR expression levels and response rates. Recent research suggests the existence of multiple EGFR isoforms. These isoforms may be an unexpected target of EGFR-directed antibodies in humans and may interfere with accurate pharmacodynamics and pharmacokinetic measurements, moderate therapeutic efficacy, and contribute to the disparity between observed EGFR expression levels and responsiveness to EGFR-targeted therapies.

~ Jane de Lartigue, PhD

EGFR in Cancer Drug Development

EGFR-specific treatments developed thus far fall into 2 distinct categories: (1) small-molecule tyrosine kinase inhibitors (TKIs) that block the adenosine triphosphate (ATP)-binding site and prevent tyrosine kinase activity; and (2) monoclonal antibodies that block extracellular ligand binding.

Drugs that target EGFR, among other pathways:
- gefitinib (Iressa, AstraZeneca)
- erlotinib (Tarceva, Genentech)
- cetuximab (Erbitux, Bristol-Myers Squibb)
- panitumumab (Vectibix, Amgen), lapatinib (Tykerb, GlaxoSmithKline)
- vandetanib (Caprelsa, AstraZeneca)

Agents under development include:
- TKI afatinib (Boehringer Ingelheim)
- monoclonal antibody nimotuzumab (YM BioSciences Inc)

Hedgehog Speeds Along
A New Drug for Basal Cell Carcinoma Emerges:
Here's What You Need to Know Now

Dubbed the Hedgehog gene (*HH*) because it caused the fruit fly embryo to develop as a spiny ball, *HH* was first identified as one of 15 genes that, when mutated, caused body segmentation defects in fruit fly embryos. The investigators went on to win the Nobel Prize, and Hh came to be recognized as an intracellular signaling ligand with import to both cancer initiation and progression.

HH initiates the Hh pathway, which plays an important role in the regulation of embryo development—from flies to humans. By acting as a morphogen, Hh generates different responses from embryonic cells, depending on its concentration. It drives developing cells to different fates, thereby controlling the formation of many organs, including the central nervous system, limbs, lungs, heart, and eyes, and the development of left-right body asymmetry. While significantly reduced in adults, recent evidence

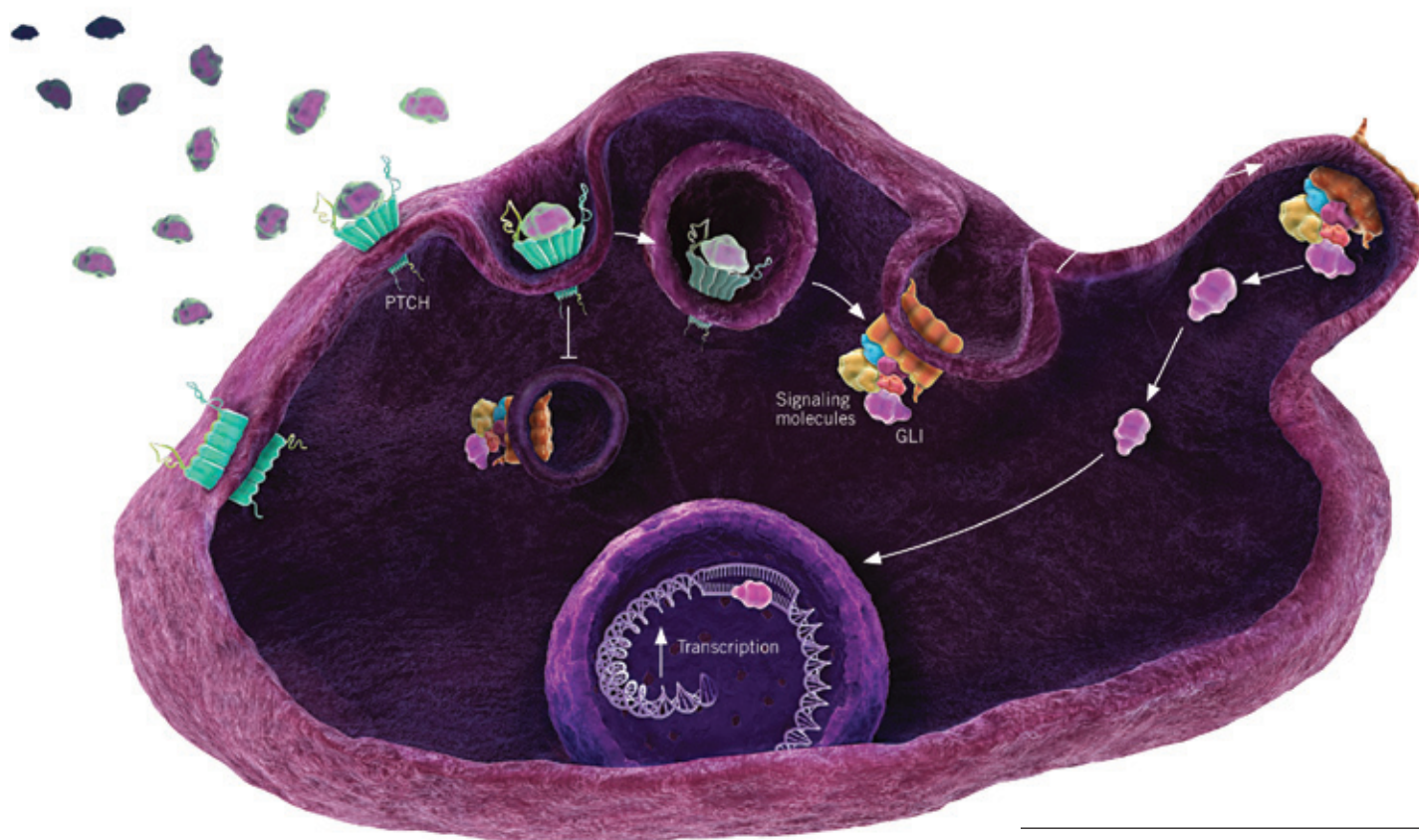> *Improper Hh signaling is thought to be involved in as many as one-third of all cancers.*

suggests that Hh activity may have important functions later in life; namely, in regulating the growth of some stem cells, including those in the adult brain, and in tissue regeneration. In general, it appears that it is critical to keep signaling "off" in most normal adult cells.

Three Hh proteins exist in humans: sonic hedgehog (SHH), Indian hedgehog (IHH), and desert hedgehog (DHH). The pathway involves the binding of Hh ligand to membrane receptors (Patched [Ptch]) on the surface of the same (autocrine signaling) or different (paracrine signaling) target cells. There are 2 Ptch receptors in humans, Ptch-1 and Ptch-2. In the absence of the Hh ligand, Ptch suppresses the expression of the downstream protein Smoothened (Smo); therefore, when Hh binds Ptch, Smo becomes activated. Smo is subsequently free to activate the Gli family of transcription factors, which initiates Hh target gene expression in the nucleus. In mammals, there are 3 Gli proteins: Gli1 and Gli2 activate target gene expression, while Gli3 represses it.

Hh in Cancer Drug Discovery

In some cancers, such as basal cell carcinoma and medulloblastoma, the contribution of active Hh signaling is clear, while in most others its relative contribution to tumor growth and behavior ranges from less clear to controversial. Overall, improper Hh signaling is thought to be involved in as many as one-third of all cancers. This number is growing with the identification of more Hh overexpressing tumors.

The first link between the Hh pathway and cancer stemmed from the discovery that Gorlin syndrome, in which affected individuals are prone to the development of numerous basal cell carcinomas (BCCs) and other cancers, is caused by an inherited mutation in Ptch-1. Accumulating evidence suggests that aberrant Hh signaling, caused by mutations in the pathway's molecular components (ligand-independent signaling) or over-expression of the Hh protein (ligand-dependent signaling), is involved in a variety of cancers. The former is implicated in BCC and medulloblastoma and the latter occurs most commonly as a result of inactivating mutations in Ptch-1 (identified in the majority of sporadic BCCs and approximately 10% of medulloblastomas), or activating mutations of Smo (found in approximately 10% of sporadic BCCs). Both mutations lead to activation of the pathway even in the absence of ligand.

The first case of ligand-dependent signaling was identified in small-cell lung carcinoma. Since then, Hh overexpression has been demonstrated in numerous other solid tumors, including gastrointestinal, pancreatic, ovarian, and breast cancers. In keeping with the "seed and soil" hypothesis that normal cells surrounding a tumor are also capable of supporting tumor growth and metastasis, Hh ligands can also act in a paracrine fashion on surrounding cells (sometimes at a substantial distance from the Hh-producing cell), and an Hh-overexpressing tumor cell may also promote tumor growth and metastasis via modification of the microenvironment.

~ Jane de Lartigue, PhD

Basal Cell Carcinoma

Courtesy Genentech BioOncology, member of the Roche Group

Arrows show the sequence of events promoting the overexpression of the Hedgehog gene.

Vismodegib Gains FDA Approval for Basal Cell Carcinoma

Given the role of Hh signaling in cancer initiation and progression, much importance has been attached to the search for ways to block its effects, and at the end of January 2012, vismodegib (Erivedge, Genentech, Roche, and Curis), a oral inhibitor of Hh, gained FDA approval for treatment of locally advanced basal cell carcinoma in patients who are not candidates for surgery or radiation and for patients with metastatic disease. Vismodegib is the first FDA-approved drug for basal cell carcinoma and was approved under the FDA's priority review program.

Vismodegib demonstrated efficacy in a single, multicenter clinical trial in 96 patients with locally advanced or metastatic basal cell carcinoma. In patients with metastatic disease, 30% experienced a partial response. In patients with locally advanced disease, 43% experienced a complete or partial response. Common side effects included muscle spasms, hair loss, and weight loss. Vismodegib was approved with a warning of the potential risk of death or severe birth effects to a fetus, and pregnancy status must be verified before treatment.

Other treatments targeting Hedgehog are under development. IPI-926 (Infinity Pharmaceuticals) is undergoing phase I trials for solid tumors. Results of a phase I trial for XL139 (Bristol-Myers Squibb and Exelixis) recently confirmed a partial response in Gorlin syndrome patients. Clinical trials of LDE225 (Novartis) were initiated in 2009, and phase I and phase II trials are currently ongoing or planned in patients with advanced solid tumors and Gorlin syndrome or in patients with pancreatic cancer. PF-04449913 (Pfizer) entered phase I clinical testing in 2010 in patients with select hematologic malignancies.

Because these inhibitors also may improve the access of chemotherapeutic agents to tumor cells through their effects on tumor microenvironment, several are also being tested as combination therapies. IPI-926 is undergoing phase II trials in combination with gemcitabine for pancreatic cancer, and PF-04449913 is undergoing phase I clinical testing in combination with dasatinib for chronic myeloid leukemia. Other proposed Hh pathway inhibitors targeting SHH (eg, robotnikinin) and the Gli family of proteins (eg, GANT58 and GANT61), as well as antibodies against components of the pathway, are also in preclinical development.

Myeloproliferative Neoplasms

The Many Possibilities of JAK Inhibition
Although Ruxolitinib Is Approved for a Rare Disorder, JAK Inhibitors May Also Have a Place in Treating Breast, Pancreatic, and Lung Cancers
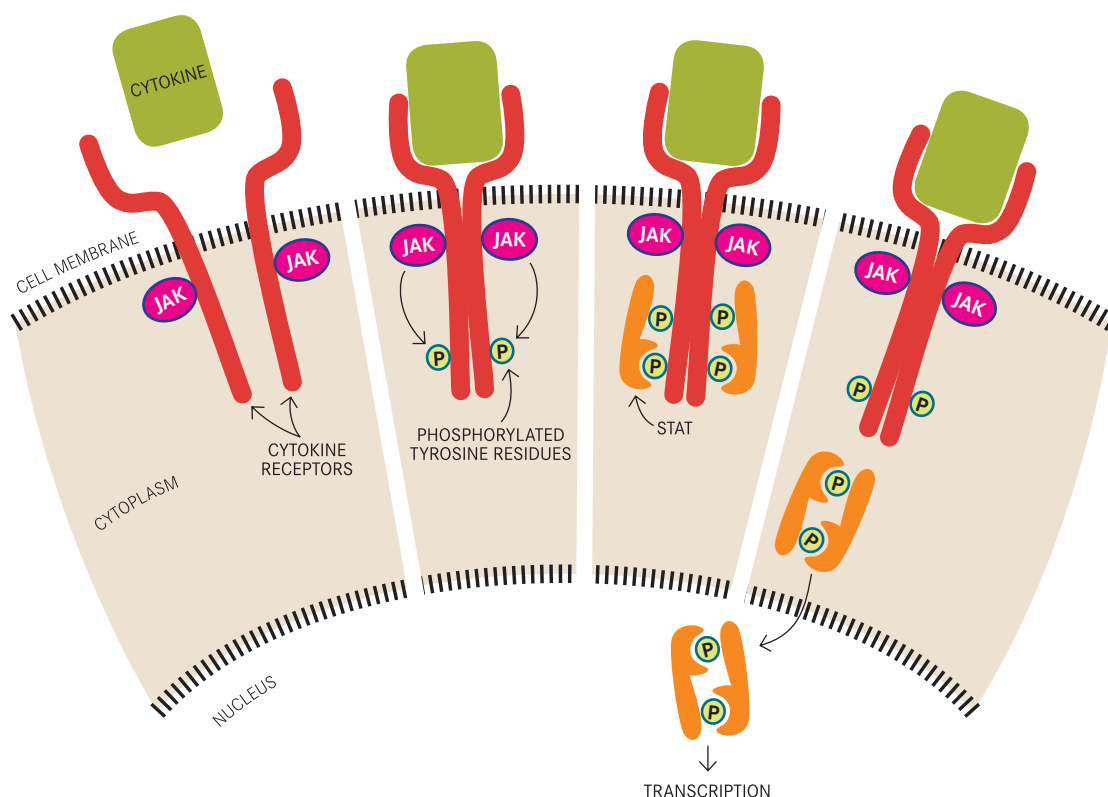
Key elements of the JAK–STAT pathway.

Found in slime molds and humans alike, JAK was once referred to as "just another kinase." Today, the JAK–STAT signaling pathway is one of the best understood of the signal transduction cascades.

In normal physiology, JAK nonreceptor tyrosine kinases are critical in the signaling of a number of cytokines and growth factors involved in hematopoiesis and immune cell functions. Among the cellular processes stimulated by JAK activation are proliferation, differentiation, migration, and apoptosis.

Because of its critical role in hematopoiesis, it is not surprising that aberrant activation of the JAK-STAT pathway has been implicated in the development of hematological malignancies, including myeloid and lymphoid leukemias. Researchers are also beginning to appreciate the role of JAK-STAT signaling in solid tumors, including breast, pancreatic , and non-small cell lung cancer.

JAK-STAT signaling is relatively simple. There are 4 members of the Janus kinase (JAK) family: JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2). Once activated, the JAKs phosphorylate their major target, the signal transducer and activator of transcription (STAT), of which there are 7. When phosphorylated, the STATs, which are transcription factors that normally lie dormant in the cytoplasm, move into the nucleus, where they bind to specific DNA sequences and drive or inhibit the suppression of target genes. This mechanism offers a means of directly translating incoming signals from outside the cell into cellular responses much more quickly than other signal transduction pathways,

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with each particular type of receptor-JAK-STAT combination driving a specific response. With 3 principal components—a receptor, a member of the JAK family, and a member of the STAT family—the pathway handles incoming signals from a number of different membrane receptors, most importantly the cytokine receptors. What is more complex and less well understood is the considerable crosstalk between JAK-STAT and other signaling pathways.

JAK-STAT in Cancer Drug Development

It wasn't until identification of the V617F point mutation in JAK2 in 2005 that mutations in the JAK gene were directly linked to the development of cancer. This defect has subsequently been shown to be important in the development of myeloproliferative neoplasms (MPNs), characterized by overproduction of blood cells. MPNs can be further characterized by the presence or absence of the Philadelphia chromosome, an abnormality in which parts of chromosomes 9 and 22 break off and exchange places. The result is that 2 genes, breakpoint cluster region (*BCR*) and Abelson tyrosine kinase (*ABL*), form the oncogenic *BCR-ABL* fusion.

Despite the initial promise of targeted therapies such as gefitinib (Iressa, AstraZeneca) and imatinib (Gleevec, Novartis) for the treatment of MPNs, the 3 Philadelphia chromosome-negative forms of MPN—myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET)—were resistant to these treatments. The *JAK2* V617F mutation has been detected in >80% of PV cases, in approximately 40% to 70% of ET cases, and in roughly 40% to 60% of MF cases. Thus, the discovery that JAK-STAT signaling is frequently perturbed in these forms of MPN was an exciting and important breakthrough.

In November 2011, the FDA approved ruxolitinib (Jakafi, Incyte Corporation) as the first drug to specifically treat patients with MF. Ruxolitinib is an oral JAK1 and JAK2 inhibitor. "Jakafi represents another example of an increasing trend in oncology where a detailed scientific understanding of the mechanisms of a disease allows a drug to be directed toward specific molecular pathways," said Richard Pazdur, MD, director of the Office of Hematology and Oncology Products in the FDA's Center for Drug Evaluation and Research. "The clinical trials leading to this approval focused on problems that patients with myelofibrosis commonly encounter, including enlarged spleens and pain." A number of other JAK inhibitors are under development **(see sidebar)**.

Although effects to date have been more palliative than curative, researchers expect that JAK2 inhibitors will become the new standard of care for patients with primary MF, as well as post-ET and post-PV MF in the near future. Complete responses have not been demonstrated in *JAK2* V617F-positive patients, and relapses occur upon cessation of inhibitor therapy.

Experts now widely believe that JAK inhibitors will make much more of an impact in combination with other targeted therapies, including, potentially, inhibitors of histone posttranslational modification (ie, histone deacetylase [HDAC] inhibitors) and immunomodulatory drugs.

JAK2 inhibitors may also play a role in the treatment of other hematopoietic malignancies and solid tumors with aberrant activation of the JAK-STAT pathway (eg, Philadelphia chromosome-negative leukemias, acute lymphoblastic leukemia, acute myelogenous leukemia, multiple myeloma, and non-Hodgkin lymphoma), and recent research suggests a possible role of aberrant JAK-STAT signaling in breast cancer.

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JAK Inhibitors in Development

The following JAK inhibitors are currently in development, with varying interim results:

• Interim updated safety and efficacy results were presented for YM BioSciences Inc's CYT387 at ASCO. While undergoing phase I and II clinical trials, TG101348 (Sanofi), XL019 (Exelixis, Inc), SB1518 (S*BIO Pte Ltd), and AZD1480 (AstraZeneca) are experiencing high dropout rates, and development of XL019 was recently halted. Finally, development of lestaurtinib (CEP-701, Cephalon) may be hampered by the superior activity and safety profiles of other agents.

• INCB028050, INCB16562 (both from Incyte), NVPBSK805 (Novartis), and R723 (Miyazaki University) are undergoing preclinical evaluation.