

# Inhibiting the Epidermal Growth Factor Receptor

*The Epidermal Growth Factor Receptor (EGFR) is a widely distributed cell surface receptor that responds to several extracellular signaling molecules through an intracellular tyrosine kinase, which phosphorylates target enzymes to trigger a downstream molecular cascade. Since the discovery that EGFR mutations and amplifications are critical in a number of cancers, efforts have been under way to develop and use targeted EGFR inhibitors. These efforts have met with some spectacular successes, but many patients have not responded as expected, have subsequently developed drug-resistant tumors, or have suffered serious side effects from the therapies to date. CCR Investigators are studying EGFR from multiple vantage points with the goal of developing even better strategies to defeat EGFR-related cancers.*

## EGFR and Lung Cancer

“When I began my postdoc in Harold Varmus’ lab at Memorial Sloan Kettering Cancer Center in 2004, *EGFR* kinase domain mutations had been discovered in lung adenocarcinoma patients,” said Udayan Guha, M.D., Ph.D., Investigator in CCR’s Thoracic and Gastrointestinal Oncology Branch. “Companies were developing drugs against *EGFR* and expecting that all patients would respond.”

Unfortunately, only approximately 10 percent of lung cancer patients responded to tyrosine kinase inhibitors (TKIs) in the United States, and while those responses were striking early on, they soon led to relapse and drug resistance. Efforts ensued to sequence *EGFR* in tumors, and multiple mutations in the kinase domain were discovered. Guha wanted to know why tumors were so dependent on *EGFR* signaling and what was happening downstream of the wild-type receptor and of the different mutant receptors, and in response to TKIs, which target *EGFR*. He began looking at patterns of phosphorylation of proteins.



(Photo: R. Bauer)

Nina Monkash, M.S., Abnilash Venugopalan, Ph.D., Constance Cultraro, Ph.D., and Udayan Guha, M.D., Ph.D., discuss lung scans.

“I started my own lab at CCR in 2011, and I continued to work on *EGFR*-dependent phosphorylation in human lung carcinoma cell lines. My lab has worked with first, second, and now third generation TKIs,” said Guha. “We are trying to discover the differences between sensitive and resistant cells, and also how the dynamics of phosphorylation change with TKI treatment. Our overall goal is to identify actionable targets to overcome drug resistance.”

Guha and his colleagues use mass spectrometry to identify phosphorylated proteins and to quantify the degree of phosphorylation as an initial unbiased proteomics screen for studying *EGFR* signaling. Using this approach, his team recently identified the protein MIG-6 as a suppressor of *EGFR*. They found it was constitutively phosphorylated on two particular tyrosine residues in cells engineered to express cancer-causing mutations of *EGFRs*; with the

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degree of phosphorylation correlated with drug sensitivity. From these initial observations, they went on to generate a series of genetically modified mice to show that mice lacking two copies of *Mig6* had accelerated lung tumor formation driven by mutant *Egfr* (See “A Brake for Cancer,” *CCR connections* Vol. 9, No. 2).

Over the years, Guha’s laboratory has used many mouse models in which mutant *Egfrs* are conditionally and selectively expressed in the lungs, so the mice develop lung tumors similar to patients. His laboratory has also generated models to conditionally express the mutant *Egfrs* in the context of heterozygous or null *Mig6*, the target of mutant *Egfrs*. More recently, they have explored using genetically modified fruit flies as screening tools. By expressing mutant *Egfrs* in the eye imaginal disc, they can distinguish functional changes as changes in the eye phenotype. “The idea is to use this model as a way to explore other targets we’ve discovered from our proteomics screen,” said Guha. “We can make these transgenic flies in two to three months, and make genetic crosses with different targets. Moreover, we’ve started treating embryos or larvae with TKIs and in a lot of cases, the mutant phenotypes are reversed, giving us a potential drug screening tool.”

In addition to cell and animal models, Guha has clinical protocols under way to study *EGFR* mutations in individual tumors and the

heterogeneity of the tumors, which is likely key to cancer’s ability to evade treatment. In a rapid autopsy protocol, tissues from hospice patients are collected within three hours of death. The team collects tissues from all sites of metastases and then does whole exome/transcriptome sequencing and proteomics to understand the tumor’s heterogeneity and how it may have affected response to treatment. “Unfortunately, tumors are continuously evolving, but perhaps we can find actionable common drivers and then either in combination or through switching single targeted therapies, we can find successful treatments,” said Guha.

In another clinical protocol, Guha and his colleagues are looking at tissues that develop resistance to the newest generation of TKIs targeted to a specific mutation of a threonine to a methionine in residue 790 of the ATP-binding pocket of the *EGFR*. The inhibitor, osimertinib, was developed because this mutation, T790M, accounts for 60 percent of the resistance that develops to the earlier, first generation of TKIs like gefitinib and erlotinib. Unfortunately, resistance develops to osimertinib, too, but is usually localized to a limited number of sites. The protocol calls for ablative surgery or radiation at those sites followed by continuation on the drug.

“In the meantime, we will do proteomic and genomic analyses, create cellular models, and try novel

therapeutic combinations so that, if resistance reappears, we will have another shot at the tumor,” said Guha. “The goal is to treat patients at different time points, but also to continuously do streamlined studies so there are some options for the patient at each step of resistance. You’d like to cure their cancer, but maybe it becomes chronic disease.”

## EGFR and Brain Tumors

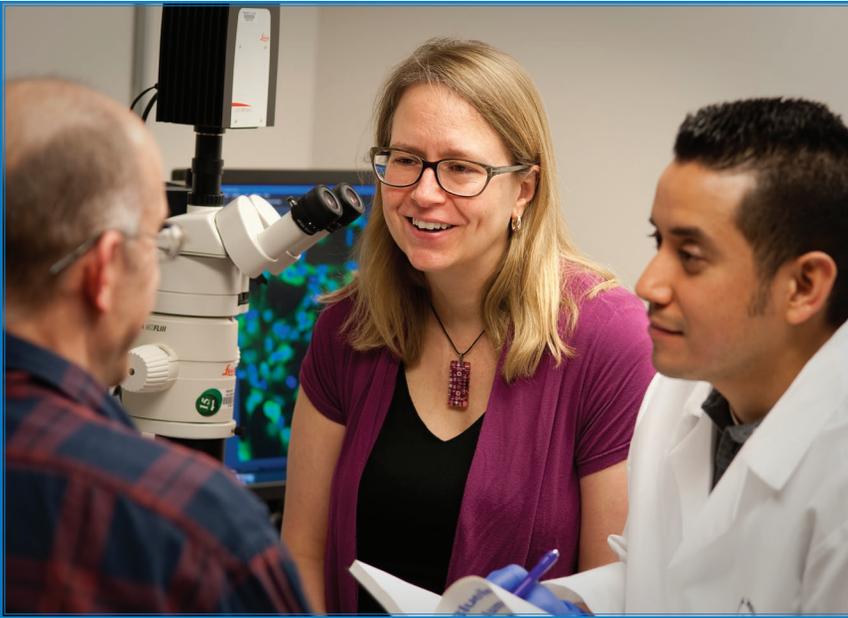
“*EGFR* is amplified and/or mutated in about half of all glioblastomas. It’s the most common alteration. In 2004, I started my postdoc with Ron DePinho in Boston at a time when multiple clinical trials were under way to test TKIs in glioblastoma,” said Jayne Stommel, Ph.D., Investigator in CCR’s Radiation Oncology Branch. “Everyone thought this would be a home run because the *EGFR* mutation is such an important alteration in glioblastoma. The TKI trials all failed and the neuro-oncologists were devastated. My postdoc project was to try and figure out why they weren’t working.”

The brain has many unique biological features, but even at the level of *EGFR* activity, clear differences between glioblastoma and other tumors exist. Unlike tumors that do respond to TKIs, such as lung cancers and chronic myelogenous leukemia, glioblastoma does not appear to have as strongly activating mutations in the *EGFRs*. Moreover, most mutated *EGFRs* in the brain seem to cooperate with the wild-type receptor, requiring coexistence in the same cells.

“We still have no idea how *EGFR* is really working in these tumors,” said Stommel.

However, in culture, *EGFR* inhibition will kill glioblastoma cells, so Stommel believes that something about the environment *in vivo* is

(Photo: R. Baer)



Jayne Stommel, Ph.D., and Carlos Tristan, Ph.D., discuss their work.

forestalling the effectiveness of TKIs. Using a novel cell-based model, her laboratory is trying to discover sensitizers to TKIs.

“We are using a system in the lab that consists of comparing biological differences between sparsely and densely plated cells. Low-density cancer cells respond to TKIs just fine, but when you plate them at high density, the cells are resistant. We see this in all cells lines—colon and lung cancer—too. It’s a very interesting system for dissecting the biological requirements for TKIs to work.”

Stommel’s cells are derived from patient tumors; they are primary

brain tumor cultures. “We are specifically looking at multiple lines,” said Stommel. “We want to find something in common for all the lines. We are not looking for a specific genomic background; we are hoping to find something useful for as many patients as possible.”

Stommel’s work is still very much in progress. She has partnered with the National Center for Advancing Translational Sciences (NCATS) to do a whole genome screen with small interfering RNAs for genes that, when knocked out, would sensitize densely plated glioblastoma cells to TKIs. Her team is currently working on the hits identified in that screen. Their work on the special properties of densely packed cells has also taken them in the direction of molecules not obviously related to cancer, namely those associated with lipid and cholesterol metabolism.

“The biology of dense cells is very interesting; not many people are studying it in the context of cell culture. Making an impact on tumor growth and sensitivity to drugs does not necessarily involve genes associated with specific oncogenic mutations,” said Stommel. “There

are multiple ways of approaching the problem, from precision medicine to targeting biological processes required for cancerous cells to stay alive.”

## EGFR and Skin

“Anybody who is interested in cancer research, cancer treatment, and patient welfare has to be interested in EGFR because it is one of the most important and successful targets for cancer treatment in several major organ sites,” said Stuart Yuspa, M.D., Co-Chief of CCR’s Laboratory of Cancer Biology and Genetics. His laboratory has been studying EGFR as part of their focus on skin development and carcinogenesis for over 20 years.

Yuspa and his colleagues started working on EGFR in the 1990s, with a lot of their work focused on the effects of EGFR downstream from RAS signaling. They found that in cells lacking functional EGFRs, tumor formation induced by the *Ras* oncogene was inhibited. Eventually, they produced a knockout of *Egfr* in mice and showed that *Ras*-driven tumors either do not form at all or, if they do, are very small.

“Typically, when you look at a signaling diagram, RAS is downstream of EGFR, so our findings are somewhat counterintuitive,” said Yuspa.

RAS, however, induces the expression of the ligands that activate EGFR, including TGF $\alpha$ , which was shown to induce skin tumors by Glenn Merlino, Ph.D., who shares with Yuspa the title of Co-Chief of CCR’s Laboratory of Cancer Biology and Genetics. “We think EGFR ligand production is required for transformation by RAS because, normally, *RAS* mutations on their own without amplification don’t drive signaling strongly enough to cause tumor formation. The signal strength has to be enhanced by activation of EGFR,” said Yuspa.

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Meanwhile, Yuspa and his colleagues have also been studying the role of EGFR in skin homeostasis and immune function. Many years ago, Yuspa decided that in order to understand deviation from normal (i.e., early events in skin cancer or epithelial carcinogenesis, in general), he first had to understand what was normal. Thus, he focused much of his attention on skin growth and differentiation and, more recently, the skin's role as an immune organ.

"Skin is the major immune organ of the body, by virtue of its size," said Yuspa. "Homeostasis of immune function in the skin is very important, and plays a role in skin cancer. In particular, we've known for many years that inflammation in the skin plays a role in tumor formation. People think of EGFR and downstream signaling as a proliferation stimulus in general, but

in the skin it has a more important function in immune homeostasis."

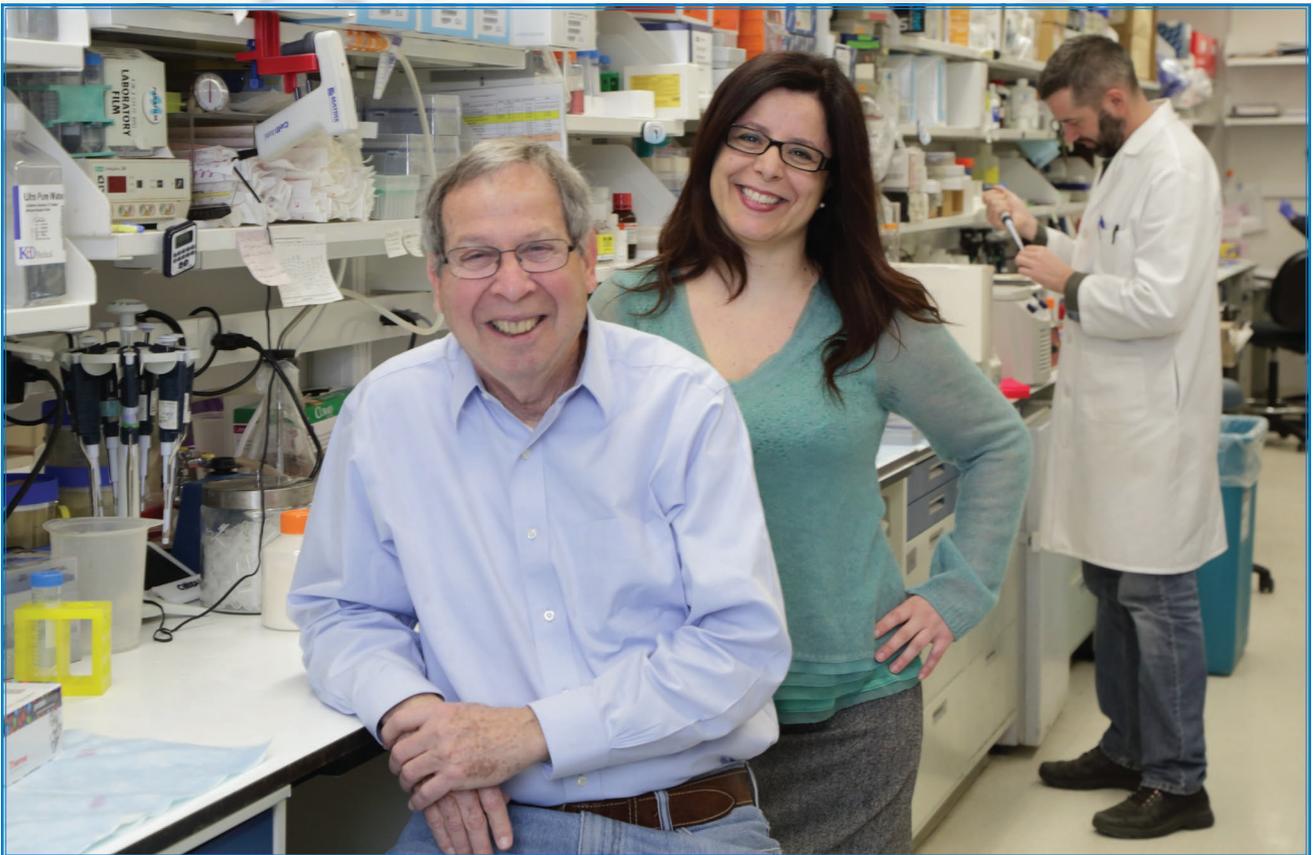
A few years ago, Francesca Mascia, Ph.D., joined the laboratory from Italy, as a Postdoctoral Fellow. During her doctoral work, she had studied immune homeostasis in keratinocytes, and had a wealth of information on cytokines and chemokines that are influenced by the status of the EGFR. Mascia, Yuspa, and their colleagues began studying the effects of EGFR inhibitors—TKIs—on the skin inflammatory response.

"Almost all the targeted cancer drugs have a skin problem as one of their major adverse effects," said Yuspa. "TKIs are a prime example. The skin response is so dramatic that it can stop patients from taking a drug or cause the oncologist to reduce the dose," said Yuspa.

To better understand the cause of the skin response, Mascia and Yuspa

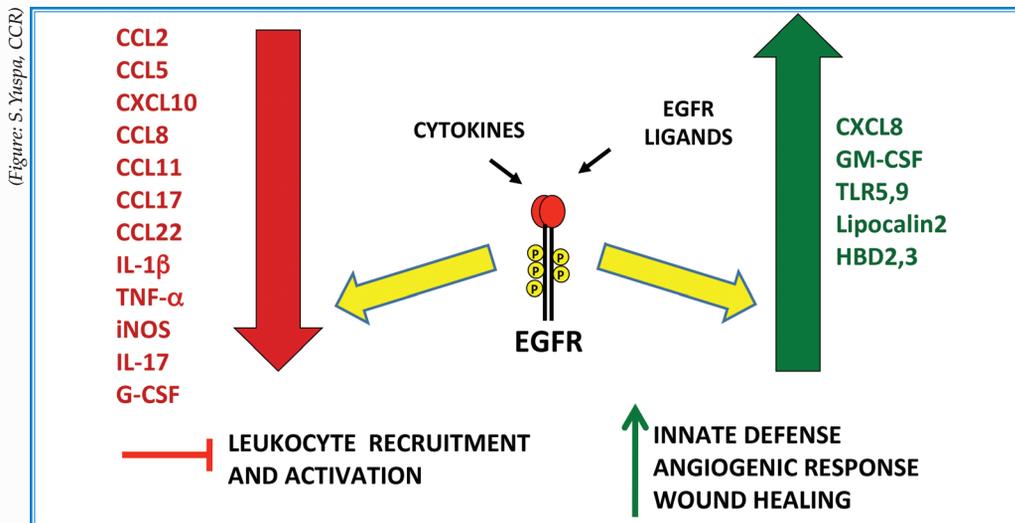
first obtained clinical samples from their colleagues Elise Kohn, M.D., formerly an Investigator in CCR's Medical Oncology Branch and now in NCI's Cancer Therapy Evaluation Program, and Seth Steinberg, Ph.D., in CCR's Biostatistics and Data Management Section. They found increases in leukocyte counts and chemokines in samples treated with the first generation TKI gefitinib that paralleled the clinical occurrence of skin rashes and pruritus.

"The clinical material was 10 years old," said Yuspa. "At the beginning of the first studies using EGFR inhibitors, many clinicians were looking for what it did to their tumor cohort. Elise had a very active ovarian cancer clinic, and I think it was an attempt to see whether or not these drugs could have an effect on ovarian cancer. We were very fortunate to have these samples and that her team had had



(Photo: B. Branson)

Stuart Yuspa, M.D., and Francesca Mascia, Ph.D.



The direct activation of EGFR in the epidermis in response to ligand release or cytokines elicits a dual response to protect tissue integrity and repair damage. A subset of innate immune effectors (green) is positively regulated while other pro-inflammatory factors are suppressed (red). Inactivation of EGFR signaling undermines this protection and can result in immune cell infiltration, barrier and differentiation defects, infection, impaired wound healing, and severe cutaneous inflammation.

a dermatologist characterize the skin response.”

In parallel, Mascia and Yuspa created a mouse model in which *Egfr* was selectively ablated in the epidermis. The mice developed skin lesions similar to those seen clinically, and before the lesions developed, the team found an upregulation of circulating chemokines and changes in blood counts that also echoed results from patient samples. Crossing the mice with mutant mice deficient in each of several immune-related factors (TNF- $\alpha$ , MyD88, NOS2, CCR2, T cells, or B cells) failed to affect the skin response, but local depletion of macrophages was partially effective.

“Whenever skin is perturbed in any way, it releases large amounts of antimicrobial peptides, cytokines, and chemokines that circulate to produce systemic effects,” said Yuspa. “And that’s really what we are seeing in patients who are on TKIs. We are seeing systemic release of a large number of cytokines and chemokines from the EGFR-depleted skin that results in infiltration of the primary cellular fighters of infection/inflammation coming back to the skin.”

Yuspa and his team are investigating avenues that could help prevent skin side effects that are associated not just with TKIs, but with other targeted therapies including MEK and VEGF inhibitors. In addition, they are pursuing evidence suggesting that part of the therapeutic effect of TKIs may be mediated via the immune system and not simply by blocking the proliferative effects of oncogenic EGFR.

“We have data pointing to an altered immune response in the tumor milieu, which may also play a role in antitumor effects of TKIs,” said Yuspa. “Some data suggest that a worse skin response to TKIs is associated with a better tumor response. In our current studies, we have preliminary evidence that in a tumor lacking EGFR, the immune environment of the tumor is altered. It’s possible that the immune response in skin is paralleled by a response in the tumor milieu that contributes to the antitumor activity. Basically, our next step is to try to characterize and understand whether the immune system is playing a

role in the antitumor activity of these drugs.”

Now in their third generation, TKIs to inhibit EGFRs are a powerful tool for fighting many kinds of cancers. Through better understanding of their biological actions, CCR Investigators will continue the effort to further improve on their therapeutic efficacy.

To learn more about Dr. Guha’s research, please visit his CCR website at <https://ccr.cancer.gov/thoracic-and-gastrointestinal-oncology-branch/udayan-guha>.

To learn more about Dr. Stommel’s research, please visit her CCR website at <https://ccr.cancer.gov/radiation-oncology-branch/jayne-m-stommel>.

To learn more about Dr. Yuspa’s research, please visit his CCR website at <https://ccr.cancer.gov/laboratory-of-cancer-biology-and-genetics/stuart-h-yuspa>.