In the mid-1990s, the miracle drug Gleevec revolutionized cancer treatment, offering terminally ill patients with chronic myeloid leukemia (CML) a new lease on life. Until then, the only weapons in the medical arsenal against cancer were the blunt and brutal triad of surgery, radiation, and conventional chemotherapy. Gleevec, which ushered in the era of molecularly targeted medicine, proved to be a broadsword with which to fight cancer. The drug was designed to block the action of a class of enzymes called tyrosine kinases, which activate signaling proteins by adding a phosphate tag to them. As their name suggests, tyrosine kinases attach phosphates to amino acids called tyrosines, and the phosphorylated proteins set off a cascade that turns cells cancerous. Salk Institute biochemist Tony Hunter discovered tyrosine phosphorylation in the late 1970s, publishing his findings in a pair of key articles in *Cell* (1) and *PNAS* (2). At the time, Hunter had nary a clue that his work would transform cancer treatment in less than two decades. “I didn’t set out to cure cancer, but that’s just the way things turned out,” recalls Hunter. Hunter’s discovery was not the only cancer breakthrough to mark the mid-1990s. When MD Anderson Cancer Center immunologist James Allison reported in *Science* (3) that the immune system can be trained to attack tumors by jamming its inbuilt brakes, it was hailed as a lapel-grabbing advance. The strategy, named checkpoint blockade, has yielded a handful of blockbuster drugs—Yervoy, Opdivo, Keytruda, Tecentriq—that have made cancer immunotherapy a frontline treatment for dozens of patients worldwide. Yet the question of why only some patients benefit from immunotherapy continues to puzzle researchers. With unbidden certainty, Allison prognosticates that an approach that combines immunotherapy with molecularly targeted drugs and radiation might help improve response rates in clinical trials. For their complementary efforts to combat a redoubtable foe, Hunter and Allison, both members of the National Academy of Sciences, were rewarded with the 2017 inaugural Sjöberg Prize for cancer research by the Royal Swedish Academy of Sciences. *PNAS* spoke with the duo to mark the occasion.

**QNAS: What drew you to the biochemical analysis of cancer?**

**Hunter:** After my undergraduate and doctorate in Cambridge, England, I stayed on as a college fellow. Then, in 1971, I followed my first wife to San Diego, where she was pursuing a postdoc in immunology. I was casting about for positions, and a colleague in Cambridge recommended the Salk Institute, which was only 5-years-old at the time. I ended up working with Walter Eckhart, who had trained with Renato Dulbecco [who won a share of the 1975 Nobel Prize in Medicine or Physiology for his work on cancer-causing viruses] and was using polyoma DNA tumor virus to study human cancer. President Nixon had just passed the National Cancer Act and money was flowing into cancer research. So I worked on polyoma virus, at first not so much on how the virus turns cells cancerous but how it replicates its DNA. Later, when my wife and I split up, I went back to Cambridge to finish my college fellowship and look for jobs in the United Kingdom. Meanwhile, Renato had left the Salk, and the Institute decided to fill his vacant lab space with junior appointments, which included myself and Inder Verma, the current editor-in-chief of *PNAS*. When I returned to the Salk, I set about characterizing the proteins made by polyoma virus that turn cells cancerous.

**QNAS: How did you fasten on protein kinases as a focus of your studies on cancer?**

**Hunter:** By 1978, we knew the proteins that polyoma virus made from the region of its DNA thought to be necessary for tumor formation. We began focusing on
the protein reported to be the most significant: the middle T antigen. Around this time, Raymond Erikson [a molecular biologist then at the University of Colorado Medical Center in Denver] found that the cancer-causing protein of the Rous sarcoma virus had protein kinase activity, an exciting finding because we knew phosphorylation could affect many cellular processes and was thus a potential trigger for cancer. So I began testing whether the middle T antigen had associated kinase activity—and it did. There were two other groups that had made similar findings, and we agreed at a Cold Spring Harbor meeting in 1979 to submit our findings on the kinase activity of polyoma virus middle T protein together to Cell. But we hadn’t pinpointed the amino acid in middle T that was getting phosphorylated. The day after we submitted the paper, I set out to do that experiment.

PNAS: The experiment had an element of *deus ex machina*. Crucial to this particular “ahah!” moment was a chemical reagent’s pH. Can you elaborate?

Hunter: For the experiment, we used 6N hydrochloric acid to chop up the $^{32}$P-labeled middle T protein sample produced in the kinase assay into single amino acids. Routinely, one separates the phosphorylated amino acids—phosphoserine and phosphothreonine—using a buffer at pH 1.9. In our system, we typically reused the buffer several times, and on this occasion I was too lazy to make fresh buffer. When I saw the experiment’s X-ray film readout, there was a new and unexpected radioactive spot. So we repeated the experiment a few days later using the same old buffer and found the same spot, which led me to suspect that this was not an artifact and that another amino acid was being phosphorylated. Further experiments showed that this amino acid was tyrosine. A few days later, I foolishly made some fresh buffer, which is when we realized what had happened. With fresh buffer, phosphotyrosine migrates on top of phosphothreonine and overlaps with it on the film; in effect, it’s masked. But when the pH of the buffer drops on repeated reuse to, say 1.7, the two phosphorylated amino acids can be distinguished on the film.

PNAS: The rest, as they say, is history: tyrosine phosphorylation was now on the map as a cancer drug target.

Hunter: Not quite. It really needed two more steps. We repeated the same experiment with the Rous sarcoma virus Src enzyme, which had been reported by Erikson to have kinase activity, to see whether it, too, phosphorylated tyrosine in my assay. Once we demonstrated that Src also had this unusual kinase specificity in vitro, and more importantly that it increased tyrosine phosphorylation in cells, we were off to the races.

PNAS: The discovery of tyrosine phosphorylation proved to be a breakthrough, partly because tyrosine kinase enzymes are “druggable” targets, as opposed to, say, Ras GTPases, which are notoriously slippery targets despite their pivotal role in cancer signaling. Did you have an inkling of the translational impact of your finding back in the late 1970s?

Hunter: Back then, it wasn’t entirely clear to us that tyrosine phosphorylation would turn out to be a central mechanism of human cancer. Renato Dulbecco told me at the time that this was going to be important, and he had very good intuition. But it would be fair to say that we weren’t really convinced. I don’t think Pharma was convinced either, partly because they were concerned it wouldn’t be possible to develop selective protein kinase inhibitors, since all kinases use ATP as a substrate. So it was not until the BCR-ABL gene fusion [an abnormal juxtaposition of segments of two different chromosomes that gives rise to the Philadelphia chromosome, implicated in CML] was discovered that the role of tyrosine phosphorylation in human cancer and the potential of tyrosine kinases as drug targets became apparent. Much later, Ciba-Geigy entered the fray to develop an inhibitor for the platelet-derived growth factor receptor tyrosine kinase—not for cancer but to prevent restenosis after balloon angioplasty—and ended up with what is now known as Gleevec. [In March 2017, the *New England Journal of Medicine* reported that the overall 10-year survival rate of CML patients receiving Gleevec was more than 83%, cementing the drug’s longstanding reputation as a miracle cure for some forms of cancer (4).]

PNAS: By some estimates, human cells harbor more than 500 kinase enzymes. Are there undiscovered drug targets among them?

Hunter: I am sure there are. There has been a growing realization that only 10% of the 500 or more kinases have been characterized in detail. The ones we have focused on were found to play key roles in signaling or discovered as mutant oncoproteins. But there is certainly the "dark matter" of the kinome [the complement of kinase enzymes in cells] that remains unexplored. With efforts to tinker with the kinome using methods like CRISPR, I think many more drug targets will emerge. Besides, there are hundreds of ongoing clinical trials of kinase inhibitors for cancer that might yield potential new drugs.

PNAS: It is now clear that merely targeting driver mutations in cancer is not always enough to thwart the disease because tumors can rewrite signaling pathways and sidestep targeted drugs. Are there ways to predict the emergence of drug resistance through approaches aimed at studying their effects on signaling?

Hunter: I would say we are not very good at predicting which signal transduction pathways may be implicated in drug resistance, but we’re improving. For instance, in B-RAF-driven melanoma, we are getting a better idea of which pathways are likely to be rewired when we treat with kinase inhibitors. If the resistance is due to a mutation in the kinase itself, a second- or third-generation drug can be developed to counter the resistance. [Resistance-conferring mutations in the ALK kinase enzyme that arise in response to the first-generation drug crizotinib, for example,
can be countered with the newer drug lorlatinib to treat some forms of nonsmall cell lung cancer.) But in other cases, we might need to target a different tyrosine kinase or use a combination of drugs that act downstream of several tyrosine kinases. Of course, the crucial element is the degree of drug-related toxicity that patients can tolerate.

PNAS: As cancer immunotherapy trials get underway, the underlying mechanisms are slowly emerging. For example, one recent CAR-T [Chimeric Antigen Receptor T-cell therapy] trial for adult lymphoblastic leukemia found that disease load at the start of the treatment correlates with response and survival rates, as well as severity of side effects. Do you think disease load might be a viable prognostic biomarker for some forms of cancer immunotherapy?

Allison: It does make intuitive sense; the bigger the tumor, the harder it is to eliminate. But it’s not entirely clear whether there are factors other than tumor size underlying the observed effect. I don’t think anyone has done a systematic study of this effect in patients receiving checkpoint blockade. One major difference between CAR-T therapy and checkpoint blockade is that the former targets one antigen, whereas with the latter we could be potentially targeting dozens of antigens. What’s more, I think it will be different for different types of cancer. That said, it is safe to say that tumor load could serve as a potential biomarker for at least some types of cancer. More importantly, there is a growing realization that immunotherapy needs to be started early, well before tumors get large, so this might circumvent the issue of using disease load as a biomarker altogether.

PNAS: Your own work on immunotherapy-related adverse events has found that clonal expansion of killer T cells in blood might serve as a potential biomarker for adverse events in patients receiving checkpoint blockade treatment for metastatic prostate cancer. Are you following through on these preliminary findings, published last year in PNAS (5), with prospective studies?

Allison: We’re not conducting longitudinal studies on adverse events, but some of my colleagues are looking at the response rates to immunotherapy over time, and the overarching finding is in favor of early treatment. So far, however, there has not been any luck in finding definitive predictive markers for prognosis.

PNAS: One of the more disconcerting reports in recent months relates to the seeming acceleration in tumor growth in small groups of cancer patients who received checkpoint blockade treatment. Can you comment on these findings?

Allison: There have been quite a few reports of accelerated tumor growth following checkpoint blockade, but these have largely turned out to be instances of pseudo-progression. The tumors get infiltrated with T cells once treatment begins, and this can be mistaken as a sign of tumor growth. If this is real progression, it’s going to be relatively rare.

PNAS: Now that immunotherapy is part of mainstream cancer treatment, scattered reports of alarming side effects, such as acute-onset type 1 diabetes, are roiling the field. Are there ways to predict such side effects, say, on the basis of patients’ genotype or metabolic status?

Allison: I have heard about these side effects, and they are quite real. It is conceivable that a complete blood work-up before enrollment in immunotherapy trials might reveal which patients are prediabetic and prone to such side effects. There are also other types of extremely rare side effects, in which the treatment starts attacking heart cells, and this kind of rare side effect has proven fatal in a small number of patients. So, once again, not everyone might be a candidate.

PNAS: Since 2012, almost a quarter of the Food and Drug Administration’s breakthrough therapy designations have gone to cancer immunotherapy drugs. Do you think the basic biology research is keeping pace?

Allison: No, I don’t. We just completed a study in which we used flow cytometry-based methods to compare the mechanisms of targeting two different antigens in checkpoint blockade. What we found is that the mechanisms and the kinds of cells that are being mobilized by the treatment are surprisingly different: there is almost no overlap, and this difference is not widely appreciated. Similarly, there is a lot of ongoing work on relapse after PD-1 blockade, and the underlying mechanisms are largely a black box at the present time.

PNAS: Part of the challenge in unraveling the mechanisms of immunotherapy stems from a lack of sophisticated tools to track the fate of the drugs and the resultant immune response: How much of the drug is taken up by tumors? Are there T cells to be turbocharged in the vicinity of the tumors? What is the identity and sequence of immune cells engaged in the response? How crucial are these tools?

Allison: Absolutely. We wouldn’t have been able to do the study that we just did had it not been for the development of CyTOF flow-cytometry [a technique that combines cell sorting with time-of-flight mass spectrometry to label and sort cells]. It’s becoming increasingly clear that nontumor cells around the T cells in tumors can influence their function as well as the effectiveness of the immune response. But we don’t really know the details of these interactions, and we desperately need more tools to study them. Right now, it’s too far-fetched to think that these tools can be used in the clinic to screen patients systematically; they are simply too expensive.

PNAS: You have won the Royal Swedish Academy’s inaugural Sjöberg prize. Do you see it as a bellwether?

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won a big Swedish prize! Either way, it’s a great honor to be selected as one of the two winners of the first Sjöberg prize.

**Allison:** I wouldn’t want to guess. At the dinner that Tony and I attended with the King [Karl XVI Gustaf] of Sweden, I later learned, there were a number of people from the Nobel committee. All I can say is that it is truly an honor to receive the very first award, and particularly to share it with Tony, because it reflects the recognition of two completely different approaches to the same problem.

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