Herceptin: A First Assault on Oncogenes that Launched a Revolution

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This year's Lasker Clinical Research Award honors H. Michael Shepard, Dennis J. Slamon, and Axel Ullrich for their invention of Herceptin, the first monoclonal antibody that blocks a cancer-causing protein, and for its development as a life-saving therapy for women with breast cancer.

More than 250,000 women in the United States are diagnosed each year with invasive breast cancer, 20% of whom will have tumors with amplification of the HER2 gene. 20 years ago, the prognosis for those 50,000 women was dismalmuch worse than the most common type of breast cancer (estrogen receptor positive), which is treated with endocrine therapy. But today, thanks to the discovery of a monoclonal antibody that binds specifically to the extracellular domain of HER2 (trastuzumab, hereafter called Herceptin), 85% of these women are expected to survive for at least 10 years (Perez et al., 2014), a spectacular reversal in outcome for these patients.

But the story does not end there. Herceptin is also effective in the 20% of gastric cancer patients whose tumors are HER2-positive. In addition, two nextgeneration versions of Herceptin, which contain modifications of the original antibody designed to improve its tumor-killing ability, have shown clinical benefit in patients whose tumors have progressed after treatment with the parent antibody. These include ado-trastuzumab emtansine (T-DM1), an antibody-drug conjugate of trastuzumab with the cytotoxic agent emtansine. T-DM1 is internalized by HER2-positive tumor cells, then the cytotoxic payload is released intracellularly after cleavage of the linker, thus providing a large therapeutic index with limited systemic toxicity. T-DM1 is approved for treatment of HER2-positive primary or metastatic breast cancer that has progressed on Herceptin (Verma et al., 2012). A second antibody-drug conjugate trastuzumab deruxtecan (DS-8201), which contains a topoisomerase I inhibitor payload, also has clinical benefit in similar patients as well as those whose tumors progressed on T-DM1. DS-8210 is also active against tumors with low levels of HER2 expression and could therefore significantly expand the number of patients who benefit (Doi et al., 2017).

Thus, this single monoclonal antibody, which on its own has had a huge impact on the lives of tens of thousands of cancer patients, has also provided an innovative spark to the entire monoclonal-antibodyengineering field by convincingly demonstrating the added benefit of conjugation to toxic drug payloads. The details of how this story unfolded reveal several key ingredients required for successful translation to the clinic: the importance of basic science in laying the groundwork for therapeutic discovery, the determination of passionate individuals in championing a project fraught with risk, and a bit of serendipity in picking the right molecule as well as a successful clinical development strategy.

It Started with Oncogenic Chicken Viruses

The world of cancer biology was forever changed when Michael Bishop and Harold Varmus reported that the oncogenic *v-src* gene in Rous sarcoma virus (RSV) was derived from the normal host (chicken) genome. That finding led to a gold rush of oncogene discovery as many labs raced to isolate the protooncogenic counterparts (c-onc's) of the growing list of *v*-onccontaining retroviruses. The hunt was further catalyzed by the fact that many of these *v*-onc's (*v-src*, *v-abl*, *v-fms*, etc.) encoded kinases with specificity for phosphorylation of tyrosine residues. Identification of the human protooncogenes was of particular interest because these would facilitate interrogation of human tumors for protooncogene alterations.

The extensive genomic databases of today were obviously not available at the time, so how were these experiments conducted? A typical approach was to screen normal human genomic or cDNA libraries with a DNA probe derived from the v-onc-containing virus using reduced stringency hybridization conditions to account for species differences (e.g., a v-onc DNA probe from chicken or mouse). Axel Ullrich and colleagues at Genentech were particularly adept at this approach.

Studies of avian erythroblastosis virus (AEV), a leukemia virus in chickens, led to a major breakthrough in 1984. AEV contains two independently expressed genetic loci (v-erbA and v-erbB), both derived from the host chicken genome. Within a matter of months, several labs, including the Ullrich group, converged on the discovery that v-erbB is derived from the epidermal growth factor receptor (EGFR). The first insight came from sequencing of EGFR-derived peptides (Downward et al., 1984), followed shortly thereafter with the cloning of the full-length human EGFR (Ullrich et al., 1984). This direct connection between an oncogenic chicken retrovirus and human growth factor signaling was a critical moment in the cancer biology field, particularly since it followed a similar connection proposed just months earlier between the simian sarcoma virus oncogene v-sis and the platelet-derived growth factor (PDGF). A clear model emerged: viral oncogenes were misbehaved variants of normal cellular proteins that played fundamental





called avian erythroblastosis virus. Independent work on chemically induced rat neuroblastomas led to the

roles in growth factor signaling. But were these insights broadly relevant to human cancer, or just curiosities? And what about the prospects for therapeutic intervention, since the tumorigenic process seemed to be an amplification of normal proliferative signals? Would oncogene-directed therapies offer any advantages over cytotoxic chemotherapy?

HER2 and the Connection to a Major Human Cancer

HER2 entered the picture through a somewhat different line of investigation (Figure 1). Robert Weinberg and colleagues at MIT, having previously identified the Ras oncogene from NIH 3T3 fibroblasts transformed by transfection of genomic DNA from tumor cells, isolated a second oncogene called neu by transfection of genomic DNA from chemically induced rat neuroblastomas. Serological characterization of several neu subclones revealed a 185-kilodalton protein not seen in parental NIH 3T3 cells that was also recognized by EGFR antisera (Schechter et al., 1984). Southern blot analysis using a v-erbB probe revealed two genomic fragments, one of which was the same size as a band seen in untransformed rat cells, implicating this as the source of oncogenic neu. The identity of the second band was unknown but raised the intriguing possibility of two EGFR-like genes. The Ullrich group clarified the story in 1985 with the cloning of the human neu counterpart using the v-erbB sequence as a probe (Coussens et al., 1985). Based on the close homology with EGFR, they proposed the name HER2, for human EGF receptor 2. Two other laboratories quickly came to the same conclusion (King et al., 1985; Semba et al., 1985).

isolation of a second oncogene called neu, a mutant allele (neu*) of the normal rat neu gene. Together, these findings set the stage for the identification of their normal human counterparts, the EGF receptor (HER1) gene, and the HER2/neu, gene respectively. A DNA probe from the human HER2 gene was then used to discover HER2 gene amplification in human breast cancer. The monoclonal antibody that led to Herceptin was isolated by inoculating mice with cells expressing excess levels of human HER2 protein, followed by humanization of the murine antibody. While spectacularly successful in its own right. Herceptin has been further improved by crosslinking to different chemotherapy drugs (emtansine or deruxtecan) and by modification of the Fc region to enhance antibody dependent cellular cytotoxicity (margetuximab).

The cataloguing of v-onc's and their human protooncogenes continued at a fast pace, with an expanding community of scientists from different backgrounds. The Oncogene meeting, established in 1985 to accelerate scientific exchange, became a mandatory ritual each July at Hood College in Frederick, Maryland. Excitement was palpable. Much of the focus was (appropriately) on mechanisms of oncogenesis, but the broad relevance of oncogenes to human cancer was a question of paramount importance. Although clear connections had been made in Burkitt's lymphoma (Ig-Myc translocation), in chronic myeloid leukemia (BCR-ABL). and in neuroblastoma (N-Myc), the role of oncogenes in common human cancers was lacking.

That all changed in 1987 with the landmark report from Dennis Slamon at UCLA that the HER2 gene was amplified in ~30% of breast cancers (Slamon et al., 1987). The experimental approach seemed remarkably straightforward: prepare genomic DNA from human tumor samples, then run Southern blots using DNA probes from the growing list of protooncogenes to look for aberrant patterns. With Ullrich's newly isolated HER2 probe in hand. Slamon struck gold. But the Slamon group took a critical next step. Early in the project, they made the strategic decision to focus their interrogation on primary tumor samples (not cell lines) and only on samples with wellcurated baseline and longitudinal clinical data. The late William McGuire at UT San Antonio was a critical partner because he had established a unique human breast cancer tumor bank that fulfilled these criteria. The Slamon team found that women with HER2-amplified tumors had a substantially worse prognosis, strong evidence that HER2 likely played a role in driving tumorigenesis.

Discovery and Development of Herceptin

The convergence of many threads of oncogene biology on tyrosine kinase signaling raised the exciting prospect of oncogenedirected therapy. At first glance, the possibility of finding small molecule kinase inhibitors would seem to be chemically tractable by exploiting the ATP binding pocket present in all kinases. But the high conservation of this domain across all kinase family members raised obvious concerns about selectivity, and therefore toxicity, not to mention the potency required to overcome the high (micromolar) concentration of ATP in cells. Indeed, the kinase-inhibitor field remained largely stagnant for another 10–15 years until the dramatic proof of concept provided by imatinib (Gleevec) in chronic myeloid leukemia.

The discovery of HER2 offered a different strategy because it was a cellsurface receptor and therefore, in theory, could be targeted by a monoclonal antibody. Indeed, the Weinberg group had already raised antibodies against neucontaining transfectants of NIH 3T3 cells and demonstrated recognition of the extracellular domain of the p185 neu protein. They went on to show, in collaboration with Jeff Drebin and Mark Greene at Harvard, that these antibodies could revert the transformed appearance of NIH 3T3 neu transfectants in culture and their growth in mice. Importantly, these effects were not observed with Ras transfectants, providing evidence for selectivity (Drebin et al., 1986).

Parallel work by Ullrich confirmed that human HER2 also played a functional role in transformation based on tumoridenicity when overexpressed in NIH 3T3 cells (Hudziak et al., 1987). This, together with the anti-neu antibody data from Cambridge, triggered the search at Genentech for antibodies targeting human HER2 that eventually yielded Herceptin. Again, the approach was straightforward. Ullrich, now working with Mike Shepard, immunized mice with NIH 3T3 cells expressing high levels of human HER2, boosted the mice with purified HER2 protein, screened sera for immunoreactivity, and prepared hybridomas from the spleens of mice with the highest titers. The winning antibody, 4D5, was selected based on selectivity for HER2 versus EGFR and on selective growth inhibition of human breast cancer cell lines with HER2 amplification (Hudziak et al.. 1989). Prior experience had demonstrated the potent immunogenicity of rodent-derived antibodies in humans; therefore, it was not feasible to test 4D5 in patients. New techniques for altering the mouse antibody by molecularly grafting the variable region sequences of the mouse antibody (which mediate antigen

binding) onto a human antibody backbone had recently been described. Shepard led the effort at Genentech to humanize 4D5 using this new approach, making important technical modifications along the way that improved speed and efficiency. The outcome was the clinical antibody Herceptin (Carter et al., 1992). Importantly, Shepard and colleagues showed that the activity of Herceptin was selective at killing cells expressing high levels of HER2, a finding that had critical implications for patient selection as well as potential toxicity against normal cells expressing HER2.

Herceptin entered clinical development in 1992 and was approved in 1998, initially for the treatment of HER2-positive metastatic breast cancer. Phase 2 studies showed objective responses in $\sim 15\%$ of patients treated with Herceptin alone. providing clear evidence of clinical activity. The most convincing results came from a randomized phase 3 study showing a 20% decrease in risk of death when given with standard chemotherapy (Slamon et al., 2001). While this approval was certainly a milestone in the history of targeted cancer therapy, the best was yet to come. 8 years later, in 2006, the use of Herceptin was expanded to include postsurgical (adjuvant) treatment of HER2positive breast cancer based on a remarkable 50% decrease in risk of relapse and a 33% decrease in risk of death (Piccart-Gebhart et al., 2005: Romond et al., 2005). These landmark studies forever changed the lives of women with HER2positive breast cancer.

Lessons for Targeted Therapy

Herceptin was the first of a tsunami of molecularly targeted cancer therapies that entered clinical practice over the ensuing 20 years. What lessons were learned from this early example? First is the importance of patient selection and the critical need for a robust, clinically validated molecular diagnostic test. Without a diagnostic test to enrich for those patients most likely to respond, the phase 3 clinical trials that resulted in Herceptin approval would have almost certainly been negative. This began with an immunohistochemical assay (HercepTest) scored between 0 and 3+, with 2+ and 3+ designated as HER2-positive. Painstaking work was required to ensure reproducibility across labs and to establish a cutoff linked with clinical benefit. This latter decision was particularly challenging because the objective response to single-agent Herceptin was too low to draw a tight correlation between HercepTest score and clinical benefit during early clinical development. Only years later, once the phase 3 results of Herceptin in combination with chemotherapy had matured, was the decision on the HercepTest cutoff validated.

Early-generation kinase inhibitors, such as imatinib in chronic myeloid leukemia (CML) and erlotinib (and gefinitib) in lung cancer, were also developed using molecularly driven patient-selection strategies. For imatinib, the molecular diagnostic assay was a test already in routine clinical use to make the diagnosis of CMLcytogenetic analysis for the presence of the Philadelphia chromosome. Although this subsequently evolved to a molecular test for the BCR-ABL fusion (by FISH or PCR), it was sufficient to ensure that all patients entered on clinical trials had cancers containing the target lesion. The clinical development of EGFR inhibitors in lung cancer followed a somewhat different path because clinical trials began before there was knowledge of EGFR mutations in lung adenocarcinoma. That discovery emerged through the detective work of several academic groups who sequenced the tumors of rare patients with extraordinary clinical responses. Despite this clear association with clinical benefit, it still took several years before EGFR-mutation testing became a routine component of every new lung cancer diagnosis. This is partly because the first FDA approval of an EGFR inhibitor was in unselected patients, but it also reflects the fact that the concept of precision medicine in oncology was still in its infancy. Today, the oncology world is radically different. Drug developers, clinical trialists, and regulatory authorities now expect a detailed plan for patient selection for every new molecularly targeted therapy that enters clinical investigation. This culture change has been further enabled by advances in DNA sequencing technology, which make it possible to profile every patient's tumor for genomic alterations using multi-gene sequencing panels.

Finally, the clinical experience with these early-generation targeted therapies has refined our understanding of why they work and, through studies of resistance, has instructed the design of new and improved, next-generation inhibitors. In the case of kinase inhibitors, resistance often develops as a consequence of second-site mutations in the drug target that interfere with inhibition, a critical finding that demonstrated these agents work by targeting a single driver oncogene. Gatekeeper mutations at the ATP binding sites for BCR-ABL and EGFR provide illustrative examples and, in both cases, have guided the development of successful next-generation inhibitors. Resistance to Herceptin can also occur through changes in the drug target, specifically by use of alternative transcription initiation sites in HER2 that generate a truncated p95 protein lacking the extracellular domain. Mutations in the PI3K signaling pathway, which reverse the downregulation of the pathway that occurs after Herceptin treatment, are another reported mechanism (Arteaga and Engelman, 2014).

In addition to its inhibitory effects on HER2 signaling. Herceptin engages host immune effector cells through the Fc portion of the antibody. Indeed, the antitumor activity of the humanized antibody was initially demonstrated in co-culture experiments of peripheral blood mononuclear cells with HER2-amplified breast cancer cells (Carter et al., 1992). Whether antibody-dependent cellular cytotoxicity is a major contributor to clinical response remains unclear. Polymorphisms in the Fcy receptor gene across the human population could, in theory, result in differential recruitment of immune cells to the tumor. While there are conflicting reports as to whether different FcyR polymorphisms impact clinical response to Herceptin, it is interesting that a phase 3 clinical trial of a next-generation version of Herceptin (margetuximab) with a modified Fc terminus designed to improve recruitment of macrophages and natural killer cells has recently shown positive results. 20 years after its approval, new insights from Herceptin continue to emerge.

We honor Shephard, Slamon, and Ullrich for their discovery of Herceptin and its impact on the lives of all women with breast cancer. Their success is a testament to the wisdom of sustained investments in basic molecular biology and animal tumor virology, over decades, that set the stage of the discovery of HER2. In addition, Herceptin has served as a paradigm for many subsequent targeted therapies, with continued learnings along the way.

DECLARATION OF INTERESTS

C.S. serves on the Board of Directors of Novartis, is a co-founder of ORIC Pharmaceuticals, and is co-inventor of enzalutamide and apalutamide. He is a science advisor to Agios, Beigene, Blueprint, Column Group, Foghorn, Housey Pharma, Nextech, KSQ, Petra, and PMV. He was a co-founder of Seragon, purchased by Genentech/Roche in 2014.

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