

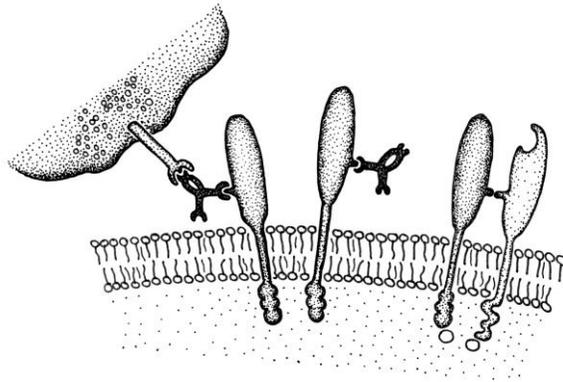
Chapter 3
of
Contrary Life and
Technical Fixes
from
malaria vaccine
to
hormone contraceptive

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Published 2015

alanwalker books, Edinburgh
<http://www.alanwalker.com>
ISBN: 0-9545173-2-6

Cancer and the antibody called Herceptin



She finished her week's work at the hospital and cycled home through the quieter back roads, wondering if she would have enough energy left this weekend to help with the tennis lessons for her grandson. Sure I will, she decided by the time she arrived home – tomorrow is another day, and if I phone my husband before he leaves work I could ask him to pick up some pizzas instead of me working to put our evening meal on the table.

Her last chore that evening was to check for any sign of a lump in her breasts. Last week – was there something there? Maybe, but why not wait until the next check? Now to the same place again. Don't fool yourself she thought, you know how it should be done.

After a sleepless night she got a message through to the office of her physician. To her dismay, within a week she found herself on the diagnostic system: a mammogram X-ray, a small biopsy sample withdrawn from the lump through a needle, the sample to the diagnostic lab for staining and examination by microscopy. She knew about this routine, she knew her vulnerability to cancer was increasing slowly. Fifty five already – I don't feel that old! Fit, healthy; surely it would be just one of those benign lumps, or at worst just a small operation will remove any problem.



When she was just six years old, starting to grow rapidly, a fault occurred in a single cell of her body. The cell was located deep in the tissues in the region of her left nipple. It was a stem cell that had arrived in that particular position when she was

developing in her mother's womb. Since then it had been quiescent, waiting for the right signal to spring into action; a type of cell dedicated to growth, development and maintenance. These stem cells would form the lobules to secrete milk and the ducts to provide it to a baby when required by pregnancy. Stem cells were plentiful and exceptionally active all over her body when she was still an embryo. They had become more concentrated in those organs and tissues that needed much maintenance. The digestive lining of her gut required constant renewal. Stem cells deep in the crypts between the absorptive projections, the villi, of the gut divided endlessly to give a daughter cell that would differentiate and move toward the tip of the outer lining of the villus. The other cell of the pair remained a stem cell, in the crypt, to divide and divide as if immortal.

Other organs and tissues of the girl's body turned over their cells at a vast rate. Bone marrow producing blood cells; skin replacing all that is worn off every day; muscle growing and repairing – a constant activity of stem cells. All adding up to a turnover of twenty five billion cells each day within the girl. Each day all these new cells slightly exceeded the number of cells that were either shed away as waste to the outside, or carefully destroyed and recycled. Despite their transience every one of these cells began like a miniature organism, self-contained and with one fundamental drive: to replicate itself by dividing in two.

That is how these cells originated. Their ancestry traces back to individual self-contained organisms. Back to the era on Earth when the most developed form of life consisted of single cells; one organism as one cell. Later, simple multicellular organisms evolved and some are still with us today, as in the species of green algae that float in pond-water. They consist of a hundred cells or so, cooperating in the shape of a self-propelled hollow ball – a fascinating sight under a microscope. To cooperate as one organism all these cells must each forego that fundamental drive to replicate as it pleases. The unitary ball will only work up to an optimum size, growth must be limited and replication confined to some special coordinated method to reproduce a new entire ball.

Multicellular organisms reaped the advantages of their more complex structures, able to exploit vacant niches in their environment. They also had to evolve ways of keeping their specialized cells under control. These cells had to differentiate precisely, do their job and then, when their internal machinery was worn out, commit suicide and suffer disposal. Their internal structure unraveled in a complex program degrading down to death. A program called apoptosis. Only cells performing correctly their allotted function were allowed to continue. Their function depended on both their development after starting as a stem cell and the cells of the surrounding tissue. The functioning of any single cell depended on signals from all its close neighbors and

some distant sources. A cellular society held in orderly balance by many constraints and feedbacks, but the difference between order and chaos was always slight.

Only the stem cells carry on, and their task is formidable. Their division requires all the genetic information in the DNA molecules of their nucleus to be copied exactly and split into two equal sets. This to be achieved in a watery cellular environment warm enough to support life and growth. Down at the molecular level all is a constant jostling of tiny molecules of water and oxygen crashing with heat energy against huge macromolecules of protein and nucleic acids. The information in the DNA, as the sequence of the bases along the molecule, is in every cell subject to this thermal agitation and liable to damage, to errors in the genetic code. Only the apparatus for repairing these constant errors maintains sufficient integrity of the code against this chaos.

A dividing cell may, in the process of replicating its two strands of DNA, its double helix, suffer one in one thousand errors in the copying of a single nucleotide base. An adenine instead of a cytosine; a thymidine instead of a guanine. An enzyme called DNA polymerase copy-edits the duplication by comparing the two strands then repairing any errors detected. The error rate may thus be reduced to only one in one million. And most of the errors are harmless anyway.

In the girl, the fault that did occur, and was not successfully detected and repaired, was to a gene called *p53*. The fault was a mutation, caused by excessive agitation of the DNA structure. An ominous event for that stem cell. This gene provides the information required for the cell to manufacture the protein p53. In turn this protein is the key to a molecular pathway that regulates the cycle of transformations the cell has to perform to divide in two. The p53 protein acts like a brake on an early phase of the cycle of division, called G1. Defective p53 protein permits the cycle to proceed faster than normal. It proceeds faster than the DNA repair mechanism can work correctly. Crisis for the stem cell ensued: its chromosomes became rearranged incorrectly and the stability of its genetic code diminished. The cell divided faster than the other similar stem cells nearby. Slowly a minute lump of cells grew from this first defective stem cell. They formed a clone of identical cells, all with the same defective *p53* gene. A renegade clone going its separate way from the normal stem cells.



At twelve years old the girl was growing fast. She was living in a boom time for her country, her city and her parents. Food was plentiful and cheap, water was clean and the air fresh. The sun shone most days. She was happy at school, whilst expected to get

higher qualifications for a better paid job than her parents had managed. Now her body did not just grow, it started secreting estrogen hormones from her newly active ovaries. She entered the path toward puberty earlier than her mother had, in harder times, and the stem cells in her incipient breasts responded precisely to this new signal. Networks of ducts grew from the divisions of these stem cells, branching like small bushes. At the tips there formed thicker lobules. Here the cells differentiated into special units, alveoli, which would become capable of secreting milk if they received the necessary hormonal signal. Twenty small mammary glands grew in each breast, along with supporting fatty tissue.

Within one of these glands remained that lump of abnormal cells, the clone with the mutated *p53* gene. It began to grow under the influence of the new hormones; slightly faster than its surrounding glandular tissue. Then, as her menstrual cycles started, these cells and all the others sensitive to estrogens, periodically divided more rapidly to start developing the glands as priming for a future pregnancy. As normal part of the cycle this growth stopped and the glands regressed.

The excess glandular cells committed apoptosis. Except for some of the cells in the *p53* clone; they now had a slightly defective mechanism for apoptosis so could survive longer. The clone grew a little faster, forming a microscopic lump of cells on the thin lining of one the glandular ducts. Resting on the basement membrane of the duct's cellular lining, the clone caused no harm.

Then one of the cells within this clone suffered another mutation. A chemical radical, a reactive oxygen species called hydroxyl, collided with the DNA of the cell. The radical was a short-lived intermediate in the complex molecular gearing of the cell's energy generators, the mitochondria. Oxygen is progressively reduced to water as the mitochondria burn the cell's fuel. Some of these intermediates are dangerously reactive. Minute amounts, during microseconds, occasionally leak out of the mitochondria into the rest of the cell.

This hydroxyl radical broke the links of a few purine bases from the deoxyribose backbone of the DNA molecule. It altered the sequence coding of a gene called *pRb*. Depurination is a disruption that cells suffer often every day; usually repaired and of no harm. The protein this gene codes for acts in a chemical pathway that influences how long the cell will live. At the loose ends of the long double strand of DNA are caps to tidy away these loose ends. They are called telomeres – resembling the sleeves on the ends of shoelaces. And every time the cell divides the telomere cap shortens. Eventually they can shorten no longer and this alarm clock signals the death of the cell by apoptosis. The telomeres were first formed by the enzyme telomerase, and the protein from the mutated *pRb* gene re-activates this enzyme so that the telomeres are lengthened instead of shortened.

The daughter cells from this single mutated stem cell then formed a sub-clone within the original clone. All its cells bore the mutated forms of the *p53* and the *pRb* genes. The constraint of the cellular clock diminished in this sub-clone so that during each menstrual cycle they proliferated a little more than their neighboring cells. They also were less likely to suffer natural disposal. These cells possessed a selective advantage, setting them out on a path evolving away from their proper function as constructors of a milk gland.

Natural death and disposal of cells that have completed their specialist task is fundamental to the life of multicellular organisms. A growing embryo is substantially sculpted into shape from a mass of more general precursor tissue. A five-fingered hand starts as a vague oval bud of a limb. As this bud expands by cell division it is simultaneously pared down between its internal rudiments of the five digits. Apoptosis pares away surplus cells one by one. Eventually nothing more than a trace of web is left between the fingers of a human hand.



The girl reached sixteen: working hard at school, nurturing an ambition to enroll for some kind of course in medical health care. With a big appetite to support her growing limbs, she gained strength through plenty of exercise. Her agility and eye for tennis helped and she acquired the build for the rigors of competition swimming. Her periods came and went; the nuisance and tenderness. The cellular lining, the epithelium, of her milk gland ducts waxed and waned with the cyclical prompting of her reproductive hormones. Pregnancy was for some far-off time in her life; maybe.

Then another reactive oxygen molecule, this time a momentary combination of nitrogen and oxygen, burst upon the DNA of one of the stem cells in her left breast. One of the cells in the progressing clone already suffering two mutants, *p53* and *rRb*. The newly mutated gene was a type described as tumor suppressor, as are *p53* and *pRb*. When functioning normally tumor suppressor genes code for the synthesis of proteins that regulate cell division and differentiation. When mutated and failing in these tasks they are likely to allow the unrestrained division of cells. A tiny tumor is likely.

It was the *PTEN* gene that suffered this mutation. The protein this gene codes for functions as an early step in a sequence of cellular signals called the Akt pathway, or protein kinase B pathway. This protects cells from premature apoptosis. The incorrect signaling of the malformed PTEN protein allowed the Akt pathway to remain permanently active. A second sub-clone formed, now with mutations to three separate tumor suppressor genes. It became even less constrained by apoptosis, with a third selective advantage over its neighboring tissues; still harmless yet more dangerous.



Delighted by her course in physical therapy at the University of Colorado Hospital in Denver, enjoying independent womanhood with her grant income, her driver license, her new friends, she decided time was overdue for a girlhood ambition. She grew up next to the splendid vista of Pikes Peak, and had travelled to the summit by the cog railway with her parents. She knew about the marathon running trail to the top, but preferred the challenge of the longer but gentler hiking trail from the north west; she was no runner. New friends from Denver, childhood friends from Colorado Springs, they all camped near Divide and next day strode out on the long trail up to the thin cool breezes of the summit.

They enjoyed a healthy day out in the clean air and sunshine; maybe with a risk of blisters from her new boots. Other risks as well: somehow, somewhere that day she suffered another mutation to that tiny tumor. It could have been caused by a gamma ray from decay of a speck of radium in dust left over from the days of the second world war, when the region was busy with many military bases. Or the same kind of ray could have emanated from natural decay of radioactive minerals in the granite mountain. She had no option but to breath in automobile and truck exhausts on the ride to the campsite. Wood smoke from the remains of a small forest fire near the trail entered her lungs and blood stream. That evening, ravenously hungry and too tired to care about a little burnt meat on the barbeque steak, she ingested toxic molecules of heterocyclic amines. So many risks in one healthy day; all exceedingly small compared to even the risk of lighting strike on the mountain. Life is full of them – sometimes one gets through.

Within the second sub-clone of the woman's breast tumor, the *HER-2* gene in the nucleus of one stem cell was hit and mutated. The normal *HER-2* gene is known as a proto-oncogene. That is, when mutated it becomes an oncogene, liable to lead to formation of a tumor and potential oncological problem. The normal protein coded by *HER-2* is an enzyme, a tyrosine kinase. It is one of those elongated molecules that sit astride the outer membrane of the cell, studding the cell with many possibilities of receiving signals from the outer environment and then transmitting them into the cell. *HER-2* protein is involved in the Akt pathway, as is the PTEN protein. When the *HER-2* proto-oncogene mutates to the oncogene form it becomes greatly over-active and far more of its protein is produced than normal – it is over-expressed. The cell membrane becomes studded with a great excess of these signaling molecules. Downstream inside the cell the natural tendency toward apoptosis is reduced.

The sub-clone of cells now had three faulty mechanisms all contributing to lessening the natural tendency of each cell toward apoptosis; and also one faulty mechanism lessening the natural ageing of the cells. But still the tumor did not amount to much physically. Hundreds of thousands of cells maybe, but the lump of cells was no wider than one fifth of a millimeter. Undetectable and causing no clinical harm it would have remained, but for several further mutations.

The woman's immune system was now robust and well experienced from the usual minor infections and her routine vaccinations. The antibodies and directly acting immune cells of her system could respond rapidly to a vast variety of threats. As importantly, they were able rapidly to acquire new highly specific abilities to fight of new threats from the outside. But the tumor cells were inside. They were mutants, but still self, of her own body. Self cells and tissues are forbidden territory for action of the immune system. For immune cells to act against any self cells is to create an autoimmune disease, such as type 1 diabetes and similar afflictions.

Antibodies and cells of the immune system are exquisitely sensitive to minute signs of difference between self cells and the character of any potential threat in the form of invading microbes or abnormal cells. The woman responded to the new appearance of these strange tumor cells with their excessive amounts of HER-2 protein exposed on their outer surface. Were they truly self? Cells of her immune system started to synthesize antibodies of the immunoglobulin G type that were sensitive to this difference in quantity. Not a strong signal for the immune system, but some response to this abnormality. These specific antibodies circulating in her blood bound with the HER-2 portions exposed on the surface of the tumor cells. In turn, they bound to specific receptors on the surface of a type of immune cell called natural killer. Some of the HER-2 positive cells were destroyed. But the prolific ability of the tumor cells, now released from four of their natural constraints, outcompeted the woman's immune system. The tumor grew a little larger.



She gained her qualifications and was pleased to find a job close to family and friends in the same hospital she had trained in, at orthopedics in Denver General. By age twenty five she dedicated her working time to getting patients back on their feet again after their bones had healed, or because their joints were old and creaking. The wasted muscles, deterioration of strength and balance, the loss of confidence of her patients, all required in her a pragmatic empathy for the frailties of the human condition. She understood our balance between advantage and vulnerability in being the only mammal

to have developed a bipedal gait. We upright apes with our bold adventure in evolution.

About that time her luck possibly turned for the worse. Within that particular benign tumor, one of many now accumulated as she grew older, yet another gene mutated. The gene now coded for an altered form of its protein such that a cascade of cellular signals stimulated excessive production of vascular endothelial growth factor. This factor stimulates new formation of blood vessels and is essential in normal healthy growth and for healing of wounds. Where there is a deep cut, slicing through the bed of fine capillaries just under the skin, and deeper down into venules and arterioles, the factor will stimulate new blood vessels to grope through the broken tissues and form a substitute network to bridge over the damage and restore the vital flow of oxygen and nutrients. The process is called angiogenesis.

The tumor, now tens of millions of cells packed now into a cubic millimeter, had been restrained in its growth by lack of its own blood supply. Simple diffusion of the molecular materials for life only works over distances of fractions of a millimeter. With its own blood supply the tumor then expanded beyond the bounds of mere diffusion. Its cells slowly spread in a bulging mass, with their increased ability to proliferate and their loss of normal specialization as glandular epithelial cells. Around and through the tumor grew a mesh of the finest capillaries that connected up at several points to the normal blood supply in the surrounding non-glandular tissue, the stroma of her breast. But still the tumor remained small and attached to the basement membrane of its glandular duct. Still benign, but only just.



At thirty two years she felt now was the time to start the family she and her husband had been wanting. Her job was secure and with good maternity leave. His job as air-conditioning engineer had improved with promotion to managing the workshop, tougher but better paid. She stopped her oral contraceptive and four months later announced the results of the pregnancy test to the delight of her husband. The balance of her hormones now changed radically with the new state of her ovaries and uterine lining. No longer did the ducts and lobules of her milk glands grow and regress each month. Now they stayed primed, growing and increasingly ready for their task of lactation.

A big task as it turned out: she bore twins. But if a smile from a baby gives the purest happiness, then these two boys were bliss. They suckled thirstily and grew well. She fed them for as long as she could manage after returning to work. Then eight months after the births she started again with the contraceptive. Maybe she would want

another child, possibly a girl, but these two kept her more than busy and greatly fulfilled. She remained unaware of the potential threat in her breast, although it was now several millimeters diameter. Like every other tissue in her body, at low but constantly present risk of mutation of a gene that might lead to formation of a tumor. Many tiny tumors grew in many tissues but came to nothing. Especially vulnerable remained all those organs with a massive turnover of cells necessary for their functions.

Another mutation in the most actively proliferating of the various sub-clones comprising the tumor occurred when she was fifty four. In the clone with five mutations. The affected cells became better able to move and push their way through the compact mass of cells and force up against the basement membrane of the glandular duct. These more active cells now had little similarity to the normal healthy cell, a stem cell in the epithelium of a milk duct. They had reverted back to a substantially less well defined, less differentiated state. A state more typical of that found in the small embryo that the woman was so many years ago. They lost their functional characteristics, their phenotype, as typical epithelial stem cells for forming ducts and glands. They transited to a flexible and labile state more like the mesenchyme cells of undifferentiated loose connective tissue. They became similar to cells in the mesoderm layer of an early embryo.

That state was enhanced by a perversion of the nature of macrophage cells of the stroma tissue of her breast. Macrophages are the universal scavenger cells of the immune system, capable of many tasks. One of them is secretion of epidermal growth factor, of use in wound healing. Certain macrophages in the stroma became associated with the tumor and this growth factor stimulated the HER-2 positive cells to proliferate. In turn the tumor cells responded by secreting another factor, colony stimulating factor, that induced more activity from the macrophages. And so on, in tight feedback.

The mutant cells became ready to break out from the confined tumor. They flattened out against the basement membrane and adhered to it strongly through molecular bindings with the laminin protein of the membrane. Then they secreted an enzyme, a collagenase, which specifically digested the collagen component of the membrane. A tumor cell, gripping the membrane with its rear part and pushing its front end down through the digested hole in the membrane, escaped. Soon more followed. Most of them got nowhere. They were lost in the stroma of the breast and engulfed by healthy macrophages, lost in the blood and swept into the spleen for filtering and disposal. A few of them stumbled into the fine lymphatic vessels that drained the nearby stroma and led to the nearest lymph node. That node was one of a series under the woman's left armpit. The loose tumor cells lodged there and continued to divide.

The tumor metastasized and began to spread. No longer benign, no longer something to live with normally. She now bore a cancer.

Some of life's risks only manifest in a chain of causes: 'for the want of a nail the battle was lost'. Any one of the mutations in the clones of cells in her cancer had a low risk. The product of a low risk multiplied by another low risk is a much smaller risk, and so on down the chain. So disasters are rare. But exposure to risk from all those minor errors of DNA replication and function, repeated over and over, year after year, with all those cell divisions in vulnerable organs and tissues, magnified the woman's lifetime risk of some form of cancer in her breasts to one in ten.

The first diagnosis was invasive ductal carcinoma. Soon followed by deeper investigations that found metastases in the lymph node. She knew well what the results might be but still the announcement shattered her. She knew well the near future: the gnawing worry, the treatment, the nausea and pain. Already she had warned her husband but could think of no easy way to tell her sons.

Then came a small glimmer of hope. The biopsy samples had been tested for evidence of that HER-2 protein in excessive amounts on the cancerous cells. Positive! But why was that biochemical fact any kind of good news?



Dr Dennis Slamon, a clinical oncologist in California, had by the early 1980s gathered a strange collection. Immersed in steel storage tanks and frozen with liquid nitrogen were small pieces of tissue removed from tumors during surgery for diagnosis and treatment. He aimed to search for clues about the nature of oncogenes; sometime in the future, some oncogenes. Slamon rode the bandwagon set rolling by a paper of Clifford Tabin in 1982. Tabin showed how a point mutation leading to the substitution of a single nucleotide base of DNA by another, a thymine instead of a guanine, was sufficient to turn a proto-oncogene into an oncogene. The smallest change possible in the DNA sequence of a gene was enough to point the protein product of the gene toward carcinogenesis. Combined with the paper from the same lab a year earlier announcing the discovery of oncogenes, this galvanized the world of cancer research done at the deepest molecular level.

When Slamon first started research on cancer as a doctoral student at the University of Chicago, money was flowing strongly from the War on Cancer campaign initiated by the National Cancer Act of 1971. Slamon had been dismayed by the bluntness of the weapons available for this battle. Their lack of specificity, inability to focus the treatment where it was needed and so avoid the dire side effects, was notorious. Slash, burn or poison – as the practitioners called the options. His

background motivated him to cut his own way through this slough with some neater blade. His grandparents had migrated to America from Syria; his father dug coal from the mines in Appalachia. When his father swapped the mines for a safer job as truck driver he then suffered a road accident that required close medical care for years. The Slamon family grew to respect greatly the profession of medicine.

From Slamon's post at the Hematology and Oncology Department of the University of California, Los Angeles, he attended a conference in Denver. Disappointed with the presentations he left early and whilst waiting at the airport chanced to meet a fellow delegate similarly returning to his lab. Slamon fell into deep conversation with Axel Ullrich. Both of them were groping for answers to similar questions; not competing, at least not yet, and with techniques and information to swap.

Ullrich was soon in Los Angeles on business for his employers, the biotechnology company Genentech. He called in on Slamon. The full story of the invention of synthetic insulin includes Ullrich as the lead author of the paper in 1977 that described the gene for insulin. The team he worked in had occupied the same building in the University of California, San Francisco, which held the competing group. That group was headed by Herbert Boyer who succeeded with his unique method of synthesis and subsequently set up Genentech. Ullrich joined up with Boyer in 1978 and went on to invent and make available a series of techniques to manipulate and clone DNA in microbes and cells – one of the midwives to the birth of biotechnology. Within ten years he returned to his native Germany and soon gained the post of head of molecular biology at the powerful Max Planck Institute in Munich.



A protein chemist, Michael Waterfield, at the labs of the Imperial Cancer Research Fund in London, needed a gene cloner. In 1983 he invited Axel Ullrich over to help him confirm a suspected link between cancer and some growth factors of the human body. Waterfield had developed the hypothesis that the receptor for epidermal growth factor is related to the protein product of an oncogene known then as *erb-B-1*; named after a cancer of chickens. This receptor molecule occurs within the outer membrane of many types of cell, with the receptor part outside and the initiator of the signal on the inside of the cell.

Ullrich helped the Waterfield team isolate the gene *erb-B-1* by working back from their knowledge of the coded protein's structure. By 1984 Julian Downward, a twenty three year old graduate student, published as first author the team paper demonstrating a pivotal discovery. They had found that an oncogene of birds was

sufficiently similar to a human gene that codes for the receptor of epidermal growth factor for it to be considered the same gene. When the gene is not working correctly, after mutating from a proto-oncogene into an oncogene, the balancing act of healthy cell division is upset by an incorrect response to a growth factor. The cell enters a pathway leading toward cancer.

Back in California, Ullrich sought evidence of other human genes that might be similar. He found a paper that Chiaho Shih had published in 1981, about a gene that would later be called *neu*. By 1984 Alan Schechter in the same laboratory characterized this gene much further. These researchers were both postdoctorals in the same lab where Clifford Tabin worked, part of the team led by Robert Weinberg at the Whitehead Institute for Biomedical Research, in the Massachusetts Institute of Technology. They had found this *neu* gene in rats, where it contributes to cancer in the brain, a neuroblastoma. But Ullrich named this gene after the second known human epidermal growth factor, *HER-2*. Later it became commonly known as *HER-2/neu*, in respect for the original discoverers.

Robert Weinberg and his group were having trouble with *neu*. Their methods they found unreliable, their results contradictory. Members of the team argued over rights to competing parts of the loose overall project on *neu*. Research drifted sluggishly into a swamp of uncertainty. These were early days in the technically tricky and uncertain pursuit of oncogenes at the molecular level. Weinberg had in his sights another idea that would have as big an impact on the global community of cancer research as his team's first big hit: the oncogene *Ras*, in 1982. He cajoled and dragged his team away from *neu*, in search of a bigger intellectual prize, possibly in reach using well established methods. They found the first tumor suppressor gene, *Rb*. Weinberg, as a biologist of cancer, sought out simpler systems for experimentation than cancers of humans. Despite missing the opportunity of clinical application offered by *HER-2*, his approach was vindicated as researchers on oncogenes and tumor suppressor genes revolutionized the field.



Cornelia Bargmann presented her recent findings on *neu* at a conference in Maryland. She had been a co-author of that paper by Clifford Tabin in 1982. Dennis Slamon was in the audience reading a different lesson from her results. Not only might this gene be important to prove ideas about membrane receptors but it might have scope for cancer treatments. Researchers in Stuart Aaronson's group at the National Institutes of Health had recently revealed the *neu* gene as extra number of its copies in cells they had cultured in the laboratory. The cells originated in tumors of the breast.

Slamon set his doctoral student, Wendy Levin, to work on his collection of frozen samples. Could this gene be found more commonly in human tumors and if so could it possibly be associated with a high receptivity to growth factors? Using assays developed in their lab from the collaboration with Axel Ullrich, she found samples of breast and ovary tumors positive for amplified *HER-2* gene. This was it! They found the signpost to a trail they had to follow to the exclusion of all else. They urgently needed many more samples.

William McGuire at the University of Texas Health Center in San Antonio was a breast cancer specialist who had gathered together a thoroughly documented archive of tumor samples. By 1987 Slamon, Levin, Ullrich, McGuire and others published in *Science* journal a paper whose title began 'Human Breast Cancer . . . ' The journal editors considered the paper important enough for full page-width banner treatment.

Few amongst the team's research peers agreed. Those who did consider it a worthwhile lead found their experimental follow-up experiments failed to confirm Slamon's results. Where had this newcomer Slamon come from, what was his specialty? Slamon was dismayed but determined to push on. He needed many more samples than from the 187 women of the first study. He knew he needed to demonstrate the repeatability of his approach and to reveal the craft skills behind the formally written methods. Within two years he and ten colleagues organized a project with samples from 688 women, with the clinical data. Methods and samples were contributed from labs in Los Angeles, San Francisco, Houston, Chicago, Alameda and Vancouver.

DNA from the tumor cells was fragmented with an enzyme, the liquid mixture was placed in a well within a slab of agarose gel and an electric current passed through the gel to move and separate the fragments accorded to their size. The fragments were then blotted from the gel onto a sheet of nitrocellulose to fix them in position. This paper was incubated in a solution of the specific *HER-2* sequence of DNA that was sought in the samples, and that had also been made radioactive. This probe would stick to any of the fragments of DNA on the nitrocellulose that were positive for *HER-2*. When the nitrocellulose sheet was layered over an X-ray film the emitted radiation exposed areas as rectangular blackish bands, in a permanent record of the experiment. The density of black in the positive bands was measured quantitatively with a laser beam. The technique is known as Southern blotting, after its inventor. The data from all aspects of the study were recorded and assessed in a protocol formally blinded to the clinical history of the women with the breast tumors.

Still Slamon and Ullrich found little support; they remained outside the peer groups of breast cancer specialists. Outsiders with an insight they knew could lead directly to the goal that everyone desired: a treatment that would attack precisely the

cancer cells and only the cancer cells. If cells in a breast tumor had excessive amounts of the HER-2 protein exposed on their outer membranes then the woman's immune system might be helped to act selectively against these abnormal cells.

The practice of immunology had recently been transformed by the invention of a method to produce antibodies with the highest possible specific avidity for the antigen that they were induced by. These were antibodies produced from the progeny of a single cell as a laboratory-bred clone. The antibodies were monoclonal. Every antibody molecule was identical in structure and activity. They lacked the slight variations of naturally produced polyclonal antibodies. Variations that diminish the power of normal antibody against antigenic foreign bodies. Ullrich and Slamon understood they had a precise antigenic target, molecules of HER-2 protein protruding from the surface of a cancer cell. These might, hopefully, react with sufficient of monoclonal antibodies manufactured to react with HER-2, to destroy the cell somehow. Or at least inactivate it.

Ullrich persuaded the Immunology Division at Genentech to produce the monoclonals against HER-2 protein. He worked hard, pushing against closed doors and deaf ears. Company policy then was to produce high value proteins to substitute for their deficiency in various diseases. This precluded work on therapies against cancer, except for a flurry of activity researching the interferon molecule as a catch-all cure for cancer. When that project collapsed Genentech disbanded their oncology staff. The style of management in those days at the firm was at best quixotic, often it was cultivated anarchy.

Michael Shepard gained Ullrich's vacant post at Genentech. Shepard knew the workings of the firm from its start-up days. To Slamon's relief he found Shepard shared his belief in the scope for monoclonal antibody therapy against breast cancer. This could become a classic of the biotechnological approach that was the core ethos of the firm – invention by rational design from basic scientific understanding to enable manufacture of high value pharmaceuticals. Slamon persistently lobbied senior managers to take up development of HER-2 monoclonal antibody as a therapy, but he was in a weak position. Without patent rights to HER-2, or funds specifically to research it, with a professional reputation resting on the still disputed evidence in two papers, and no long-term experience as a breast cancer specialist, he was trapped. To escape he relied on utter faith in his proposition and energy to carry it through all barriers.

By 1989 Slamon had Bill Young, vice-president for manufacturing at Genentech enthusiastic for the project. Their first problem was with the monoclonal antibodies. The original invention uses as its starting point mice or similar laboratory animals. First the mice are made immune to the molecule, the antigenic protein, that

the monoclonal antibody is required to react against. In this case HER-2 protein, produced in pure form by the recombinant protein technique that Genentech had brought to manufacturing process. Then those white blood cells that synthesize natural antibodies, the B-lymphocytes, are removed from the mice. These cells are short-lived but they can be immortalized. This is done by inducing them to hybridize with cultured cells derived from a myeloma, a cancer of the tissue that produces B-lymphocytes. The immortalized hybrid cells are then selected for those producing the most potent antibody. A line of cells derived from a single one of these selected cells is then cultured so that it divides repeatedly. Thus a clone is produced. When this is cultured in bulk the monoclonal antibody is secreted into the growth medium, from where it is purified for use.

But the antibody came from a mouse. Infused into a human as a treatment it would soon induce an immune reaction to its non-self nature. A reaction that could be fatal.

Slamon and Young searched for a molecular biologist who might know a way to make this mouse molecule mimic a human molecule. To humanize it was the challenge. They found Paul Carter at the Medical Research Centre in Cambridge, England. Carter achieved this wizardry of genetic manipulation by replacing certain bases of the two mouse genes that code for the antibody against HER-2. He used equivalent sequences of bases from the relevant human antibody as replacements. Antibody molecules are formed as two identical halves like a pair of bent rods joined to form something like a Y shape. It is at the tips of the two upper branches that the antibody locks onto its specific antigen molecule. Only the very tips remained as mouse origin in Carter's humanized HER-2 monoclonal antibody. A human immune system would accept the manufactured molecules as if genuinely human origin. Now the basic research scientists had invented a highly novel therapy of exciting potential.

The technologists and managers, however, faced more intractable problems – political and economic ones. Genentech was enduring lean business by the end of the 1980s, with only two products to sell for profits large enough to finance all those expensive research projects. In 1990 the Switzerland based company Hoffman La Roche bought a majority shareholding in a deal negotiated with Robert Swanson, Genentech's co-founder. To make any profit from HER-2 antibody therapy they needed to overcome widespread skepticism from oncologists about use of a therapy seemingly derived from laboratory mice. Many of the skeptics also knew that the application of the therapy would be limited to the thirty percent of women whose breast tumors were positive for HER-2 in excessive amounts. Breast cancer is one of the commonest types, but this restriction of the potential market had to be weighed against the high costs of this novel and specialized manufacture. Clinical trials would be protracted, complex,

highly expensive, and possibly contentious. Eventually, in 1998 the Food and Drug Administration approved registration of the new pharmaceutical with the generic name trastuzumab, and brand name Herceptin.



Herceptin, in solution containing the humanized monoclonal antibody that is specific for the human epidermal growth factor receptor 2, is infused into women from a drip into a vein. When the Herceptin diffuses from the blood stream through to the cancerous cells with their abnormally high numbers of HER-2 molecules, (20,000 instead of the normal 2,000 per cell) there are several separate ways it works.

A molecule of HER-2 protein is accompanied on the surface of the cell by other members of that biochemical family: HER-1 to HER-4. These others are receptive to known growth factors and HER-2 is only active when joined to, dimerized with, another family member. This pair normally acts to transduce the external signal to the nucleus of the cell. The receptor protein receives a molecular signal from outside the cell, and then it emits another series of signals within the cell directed at the nucleus. The pathway is double: one includes the Ras to MAPK signaling for cell proliferation response; the other includes the P13-k to Akt signaling for a cell survival response.

Herceptin can attach to HER-2 in a docking that blocks the dimerization with one of the other HER receptor molecules, inactivating both pathways. Or Herceptin may attach to HER-2 with the tips of its upper Y branches whilst exposing the stalk of the antibody, the Fc portion, to the woman's immune system. White blood cells such as the natural killer cells and macrophages have receptors for the Fc portion of the antibody. When there is such a docking between the immune cell and the antibody, the immune cell blasts out a shot of enzymic granules to destroy rapidly the cancerous cell. This is known as antibody dependent cell-mediated cytotoxicity; one of the body's big guns against threats of disease. Threats from viruses, bacteria, cancer cells, parasites. Herceptin provided a new way to aim and fire this gun.



Cancer has been with us since before we evolved into humans. It will remain, but researchers and doctors diminish its threat step by little step. Herceptin and all the other monoclonal antibody therapies that followed on from that seminal invention were a bigger step than most. These are steps forward amidst steps backward. We live in a world complex enough through its myriad natural opportunities and threats to our

survival and success. Then we make it even more complex with our inventions. In technologically advanced societies we all are caught in a web of complexity. Only those few peoples in the remotest regions of the Americas, Africa, Asia and Australia who still live as hunter-gatherers are free of our technological bonds and constraints.

Our reproductive biology is now starkly different from what it was a long time ago, and the share of that difference is borne almost entirely by women. Anthropologists working amongst hunter-gatherer peoples have recorded a typical reproductive life between menarche at age 16 to onset of menopause at age 48. First pregnancy is likely to be at age 20, followed by 5 more pregnancies to age 40, with lactations each lasting 2 years. During that time of motherhood there will be about 145 menstrual cycles, each time with the rapid activity of the stem cells in the breasts and womb in preparation for pregnancy.

A woman elsewhere, typically in an industrialized country where she enjoys good diet, low physical stress, effective health services and access to contraception, will experience a different reproductive life. Menarche at age 13 will be followed by menopause at 52. Two pregnancies will start from age 30 and have 1 year of lactation each. Potential menstruations add up to 440. Thus it is that modern woman is exposed to three times more divisions of reproductive stem cells than her hunter-gatherer sister. The greater the division rate the higher the risk of reproductive cancers.

I am distressed to write about this cruel irony. In my country just five generations ago most women endured lives of hard physical labor, many pregnancies and high mortality of their infants. The industrial revolution, social reform, legal rights for women, available contraceptives, and modern medicine lifted these burdens. However, that release brought with it increased risk of this frightful disease.

There is no plausible change in life-style that will reverse this dismal calculation, no going back. There could possibly be a technological improvement. The specific and most potent invention leading to this changed state was hormonal contraceptives for women. The original formulations were designed to mimic the natural menstrual cycle as it proceeds without pregnancy. That design derived from long experience of using similar hormones to treat menstrual problems and reduce risk of miscarriage. Perhaps there is scope for the hormone manipulation involved in contraception to mimic more the conditions in which we evolved.



She was approaching her sixtieth birthday when her sons approached their father with the idea of a splendid birthday party, a very particular celebration. Great idea he agreed: a great big joyous celebration of life. She had grasped for the Herceptin

therapy. She endured the side effects of the chemotherapy required as an adjuvant whilst the monoclonal antibodies slowly did their job of demolishing the shreds of metastasized tumors left after the main tumor was surgically removed. She regained her fitness; she still enjoyed an easy game of tennis and her weekly swim. She had negotiated with her head of department a new part-time post organizing the teaching of her profession. It was time for a new life and a big party. All her family was there, many colleagues, neighbors and friends from afar. No presents, but her sons gave her a magnificent bouquet of flowers. 'Hey you guys, the flowers are lovely but how about some grandchildren for me to pamper? Maybe even a granddaughter or two – we need some more women in this family of ours!'

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