Venture science: climbing the ladder to telomerase, cognitive therapy and *in situ* hybridization

Joseph L Goldstein

Lasker Awards are given to scientists who open new fields of biomedical research by carrying out bold, high-risk experiments that have a high probability for failure. But if the experiments are successful, the rewards for advancing science and medicine are mighty. In many respects, this type of science resembles venture capital, a phenomenon in which a small group of investors puts up money to finance a start-up business that has no previous track record but has daring ideas.

Venture capital had its origins in 1957 when a professor at Harvard Business School, Georges F. Doriot, convinced several of his Boston colleagues to invest in a start-up computer company founded by two MIT engineers with no business experience. Doriot named the start-up Digital Equipment, which became the anchor company for Boston's famed Route 128 and the first of Doriot's launch of more than 200 start-ups. Digital Equipment soon became IBM's biggest competitor, producing the most popular mini-computers in the 1970s and 1980s. Doriot's initial $70,000 investment in 1957 had a market value of $400 million in the early 1970s—the best return on money ever seen at the time.

The *modus operandi* of Doriot and the early venture capitalists—'no risks, no rewards'—was a heretical idea to Wall Street 35 years ago. But without 'no risks, no rewards', there would be no Apple Computer, no Microsoft, no Genentech, no Google and even no Starbucks.

The phenomenal financial successes of venture capitalists have spawned a new endeavor called venture philanthropy. In contrast to traditional philanthropy, which typically supports projects that have a low risk of failure, venture philanthropy operates on the venture capital philosophy of 'no risks, no rewards'. The Georges Doriots of venture philanthropy are people like Bill and Melinda Gates, who take ultra-high risks, back many daring schemes, assess them critically and then eliminate the failures as quickly as possible. This steely approach enhances the chances that the Gates Foundation's high-risk focus on poor-country diseases will ultimately produce new vaccines and more effective medical delivery systems that are essential for beating third-world diseases. *The Economist* has coined the term "Billanthropy" to refer to this new type of venture philanthropy.

Puryear's 'Ladder for Booker T. Washington'

Like venture capitalists and venture philanthropists, venture scientists are perpetually involved in a struggle to climb to the top of the ladder. The struggles to reach the top in science or in any endeavor are powerfully illustrated by a sculpture in the Modern Art Museum of Fort Worth (**Fig. 1a**). Created by the contemporary African-American artist Martin Puryear, this sculpture is a tall wooden ladder (36 feet high) suspended by invisible wires, enabling it to float from the ceiling of the museum's atrium. Puryear constructed the ladder from a single oak tree. The bottom rung is 2 feet wide, and top rung is 1 inch wide. There are about 100 rungs, most of which are at top and not visible in the photograph. The narrowing of the ladder toward the top creates a distorted visual perspective that evokes an illusionary goal that is unattainable. It is easy to get both feet on the first rung of the ladder, but it is an almost impossible struggle to reach the top. Opportunity narrows at the highest reaches.

Puryear created this work to symbolize the struggles of Booker T. Washington, who was the dominant African-American figure in the US in the early 1900s. Booker T. Washington was born into slavery in 1856, was freed at age 9 and, by age 25, had established the Tuskegee Institute in Alabama as a trade school for African-Americans. His autobiography, *Up from Slavery*, is still read today, more than 100 years after it was written. Harvard University conferred an honorary degree on Washington in 1896 when he was only 40 years of age.

Ladder for Venture Scientists

Puryear's ladder can be thought of as a universal metaphor for everyone who struggles to do something worthwhile. But there is another way to think about the ladder that may be more relevant to scientific discovery. If we turn Puryear's ladder upside down (**Fig. 1b**), now the difficult challenge is *not* at the top, but at the bottom—getting your little toe on the first rung. The venture scientist achieves this goal by doing a bold experiment that opens a new field, but no one recognizes it as such at the time. For a few years, the venture scientist has the first 94 rungs of the ladder all to herself or himself until she or he reaches the ninety-fifth rung (fifth rung from the top in **Fig. 1b**) where there is now room for other scientists to jump on the bandwagon and join in on the excitement of a new venture. This is the 'tipping point', when a new field of science or medicine explodes with widening opportunities and becomes recognized as worthy of the Lasker Award. In terms of venture capital and venture philosophy, the ninety-fifth rung is the tipping point when the stock price starts to hit the roof and when thousands of human lives begin to be saved.

Lasker Basic Award: venture science in action

This year's Basic Award is given to three scientists for their prediction and discovery of telomerase, a remarkable RNA-containing

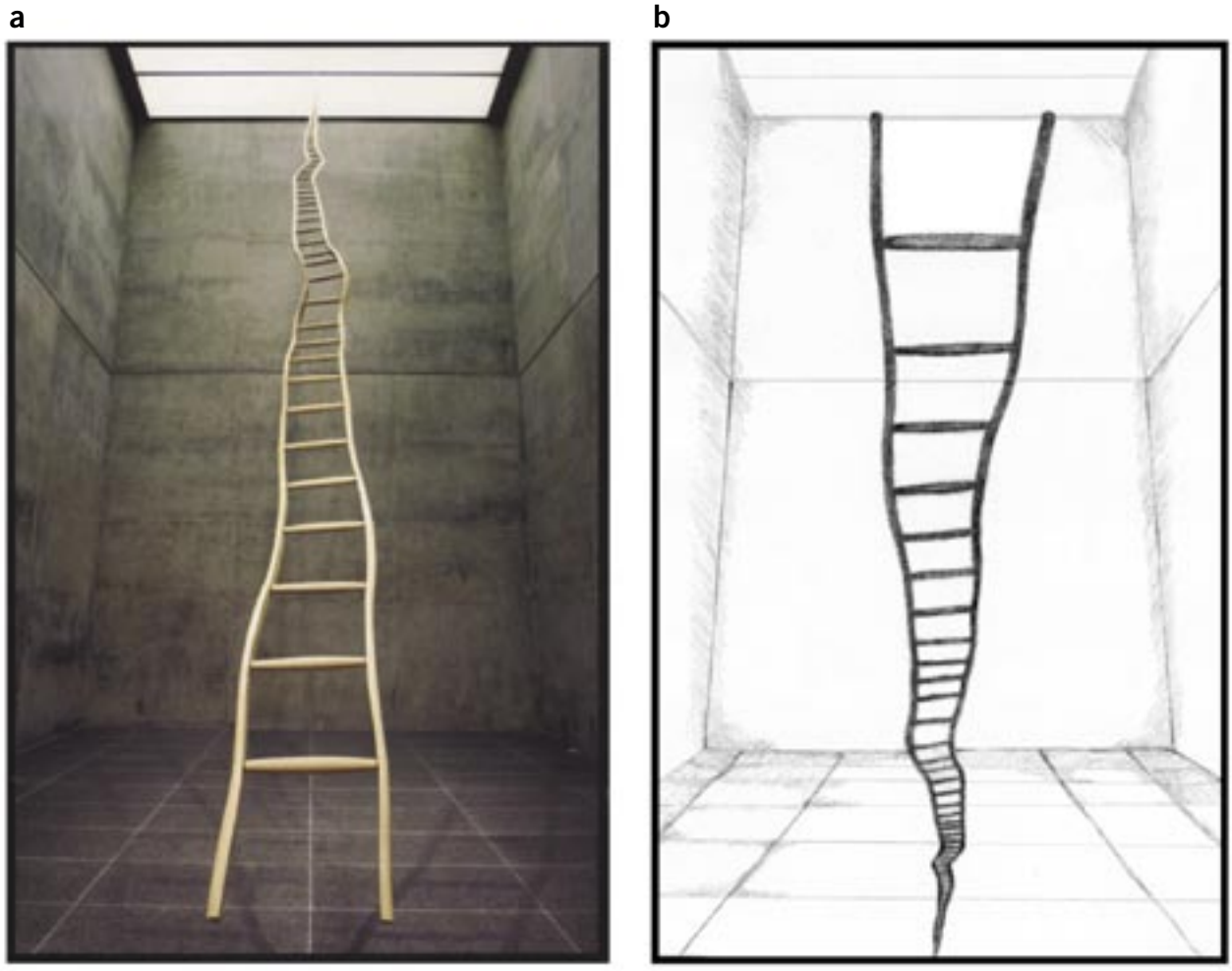

Figure 1 A climb to the top. (**a**) Martin Puryear: *Ladder for Booker T. Washington*, 1996. Collection of the Modern Art Museum of Fort Worth. Gift of Ruth Carter Stevenson, by Exchange. Reproduced with permission. (**b**) *Ladder for Venture Scientist*. Puryear's *Ladder* has been turned upside down in a drawing by Nancy L. Heard.

enzyme that synthesizes the ends of chromosomes, protecting them and maintaining the integrity of the genome. The three honored scientists are: Elizabeth H. Blackburn (University of California, San Francisco), Carol W. Greider (Johns Hopkins University School of Medicine) and Jack W. Szostak (Massachusetts General Hospital and Harvard Medical School).

Telomerase has profound implications for two fundamental processes in mammals—aging and cancer. If a cell lacks telomerase, the ends of the chromosomes (telomeres) progressively shorten with each round of cell division, eventually eroding the telomeric DNA such that the telomeres no longer protect the chromosomes from sticking to one another. The resulting chromosomal fusions and other structural abnormalities lead to cell death. On the other hand, abnormal activation of telomerase in a somatic cell can promote its growth, leading to immortality and oncogenic mutations. About 85% of all human cancers are associated with elevated telomerase activity. Inhibition of telomerase provides an exciting target for cancer therapy.

The details of the telomerase story are recounted in the article by the three prize winners in this issue of *Nature Medicine*. In brief, the discovery unfolds in four chapters, spanning a 15-year period from 1975 to 1990. Chapter 1 began when Blackburn, then a postdoctoral fellow in the laboratory of Joseph Gall (recipient of this year's Lasker Special Achievement Award; see below), sequenced both ends of an extrachromosomal gene in the ciliated protozoan *Tetrahymena* and discovered a sequence of six nucleotides, tandemly repeated 20–70 times. In 1976, this type of tandem repeat had never been seen before, and its identification raised the question of whether it was a peculiar property of an unusual amplified gene in a ciliated organism far removed from the main line of eukaryotic evolution, or rather, was it an authentic telomere sequence that would have universal relevance to the chromosomes of humans and other higher organisms.

Chapter 2, between 1980 and 1984, involved a collaboration between Blackburn and Jack Szostak. Szostak was attempting to clone genes on a single linear molecule of DNA in yeast cells. Unlike circular plasmids, the linear plasmids were unstable and would not replicate even though they contained an origin of replication and a centromere. When Szostak added Blackburn's *Tetrahymena* repeat sequences to both ends of his linear yeast plasmid, the chimeric plasmid replicated in stable fashion and expressed its encoded marker gene. Szostak and Blackburn had now developed the first functional assay for telomeres. When Blackburn and Szostak sequenced the newly replicated plasmid, the results were totally unexpected—the replicated plasmid was longer than it should have been; the yeast cells had actually added many copies of a new type of repeat sequence onto the end of

the *Tetrahymena* repeats. This discovery led Szostak and Blackburn to predict that the yeast cells contained a telomere-synthesizing enzyme—soon to be called telomerase.

Chapter 3, between 1984 and 1990, involved the challenging job of setting up an assay for the hypothetical telomerase. Any putative telomere-synthesizing activity detected in crude extracts of tissues would have to be distinguished from the multiple nonspecific nucleases that degrade any newly made nucleic acid and from the more abundant DNA polymerases that replicate the middle of chromosomes. The enzyme was purified by Greider, who began the work in Blackburn's lab as a graduate student, and who completed it several years later as a junior scientist at Cold Spring Harbor Laboratory. Greider and Blackburn discovered that the telomerase was a multisubunit enzyme composed of both RNA and protein components. The most unique component—the one Greider purified and cloned—is an RNA molecule containing a built-in template that ensures that the catalytic component of the enzyme adds the correct repeat sequence to the ends of the chromosome. To prove that the built-in template of the RNA subunit dictates the sequence of the telomeric repeats, Blackburn mutated the template sequence and showed that the resulting telomeres corresponded to the new sequence. The catalytic component of telomerase, a reverse transcriptase-like enzyme called TERT, was purified and cloned by Tom Cech's laboratory in 1996. Telomerase also contains several regulator proteins essential for its function.

Chapter 4, the last chapter in the discovery story, occurred when Szostak and his postdoctoral fellow Victoria Lundblad set up a genetic screen in yeast to identify essential components of the then-putative telomerase enzyme. The first mutant, published in 1989 and called *EST1* for "ever shorter telomeres," had a profound phenotype. Telomeric DNA gradually disappeared, chromosomes became shorter with each cell division, and the cells underwent senescence as their telomere reserve was depleted. These findings provided the first experimental evidence linking the length of telomeres to the aging of cells.

Later work by Lundblad showed that the protein lacking in the *EST1* mutant was one of the accessory regulator proteins in the telomerase complex. Another EST mutant with a similar phenotype, *EST2*, turned out to lack the catalytic component of telomerase—the same TERT subunit that Cech had purified, providing a neat genetic validation of the complex biochemistry pioneered by Greider and Blackburn.

The availability of recombinant reagents for each of the various RNA and protein components of telomerase allowed hundreds of scientists to enter the field in the 1990s, which has led to exciting new observations concerning the role of telomerase in aging and cancer.

Lasker Clinical Award: slow climb up the ladder

Disorders of mental health are a major medical problem in the world today. As many as 20% of the population in the US and UK has depression or a serious anxiety disorder. This year's Clinical Award is given to Aaron T. Beck (University of Pennsylvania School of Medicine), the scientist who developed the theory and practice of cognitive therapy, which has transformed the understanding and treatment of many psychiatric conditions, including depression, suicidal behavior, generalized anxiety, panic attacks and eating disorders.

More than anyone else in the past 50 years, Beck has changed the way psychiatry is practiced. His cognitive therapy approach is a radical departure from the psychoanalytic approach of Sigmund Freud, which dominated the field of psychiatry in the first half of the twentieth century. In Freudian psychiatry, the focus is on the unconscious, and the psychiatrist takes a passive role with patients: he or she relates the patients' mental abnormalities to early childhood experiences with their parents. In the Beck approach, the focus is on the conscious, and the therapist takes an active role with patients: he or she explains to the patients that their dysfunctional behavior is caused by a distorted way of thinking shaped by underlying beliefs that stem from a biased and exaggerated interpretation of events. For example, depression-prone individuals have beliefs such as "If I can't succeed at what's important, I'm a failure." The therapist then works directly with the patients to help them interpret their biases, beliefs and experiences more realistically. Cognitive therapy is often referred to as 'talk therapy'.

Like all radical transformations in clinical medicine, acceptance of Beck's ideas (originally proposed in 1967) was slow in coming, but after 35 years they have now been incorporated into the mainstream of psychiatry, owing largely to the repeated demonstration in multiple controlled clinical trials by Beck and others that patients treated by cognitive therapy recover more frequently than those treated by less direct approaches.

In the US, suicide is the eleventh leading cause of death, and in people between 15 and 44 years of age, it ranks third. Worldwide, more than 1 million people kill themselves each year. One of the important practical benefits from Beck's research has been the creation of the Beck Depression Inventory. Widely used for quantifying the severity of a patient's depression, the questionnaire consists of 21 multiple-choice questions composed of items relating to depression symptoms (hopelessness and irritability), cognition (guilt or feelings of being punished) and physical symptoms (fatigue, weight loss and lack of interest in sex). This Inventory, together with the Beck Suicide Intent Scale, has provided a simple and invaluable tool for identifying patients at high risk for suicide and allowing intervention with short-term cognitive therapy to reduce the likelihood of suicide attempts.

Special Achievement Award: a venturous experimentalist

This award, inaugurated in 1994, is given periodically to honor a scientist whose lifetime contribution to biomedical research is universally admired and respected for its creativity, importance and impact.

This year's Special Achievement Award honors Joseph G. Gall at the Department of Embryology of the Carnegie Institution in Baltimore. Gall is honored for an exceptional 57-year career in science—as a founder of modern cell biology and the field of chromosome structure and function, a bold experimentalist, the inventor of *in situ* hybridization, and an early champion of women in science.

Gall is the quintessential nuclear biologist: his research explores virtually every aspect of nuclear structure and function. Throughout his career, he has displayed awesome technical courage in solving problems that required the use of multiple organisms (snails, protozoa, ferns, flies, frogs, mice) and the development of new technologies based on chemistry and physics. Arguably the most influential of Gall's discoveries was the development in 1969 (together with Mary Lou Pardue) of the technique of *in situ* hybridization, which allows scientists to localize DNA and RNA to specific regions of the cell. The original procedure used a ^{3}H-labeled ribosomal RNA probe that was hybridized to squash preparations of *Xenopus* oocytes that had a marked amplification of ribosomal RNA genes. Probes of higher specific activity were soon developed, permitting localization of single copy sequences. A major advance came with the advent of fluorescent probes (fluorescent *in situ* hybridization or FISH), which greatly simplified karyotype analysis by allowing individual chromosomes to be 'painted' in different colors.

Among Gall's other fundamental contributions to nuclear biology are early observations on the structural organization of the nuclear pore complex, discovery of satellite DNA, discovery of gene amplification, and his identification of the function of the mysterious Cajal

body (first identified over 100 years ago) as a nuclear organelle in which all forms of RNA are transcribed and processed. As mentioned above, Gall also played a key role in Chapter 1 of the telomerase story. He discovered that *Tetrahymena* has two nuclei—a micronucleus that contains normal chromosomes and a macronucleus, the chromosomes of which are fragmented into thousands of small pieces of DNA, each encoding the same ribosomal RNA gene. The high abundance of this extrachromosomal gene made it possible for Blackburn and Gall to purify the molecule and sequence its tips, which turned out to be the tipping point in the telomerase story as it subsequently unfolded.

Gall is revered for his scholarly approach to science, his great sense of history, and his early championship of women in science. Joan Steitz was stimulated by Gall to a career in the biological sciences as an undergraduate at the University of Minnesota, where she worked in his lab. Three of Gall's former students or postdoctoral fellows have followed in his footsteps and served as President of the American Society of Cell Biology—Mary Lou Pardue, Susan Gerbie and Elizabeth Blackburn. Gall himself served as the Society's sixth president (1967–1968) and received its highest honor in 1983, the E.B. Wilson Award. Gall is also the recipient of the American Association for the Advancement of Science Lifetime Mentor Award in 1996.

Joseph L. Goldstein
Chair, Lasker Awards Jury

Lasker Award recipients receive an honorarium, a citation highlighting their achievement and an inscribed statuette of the Winged Victory of Samothrace, which is the Lasker Foundation's symbol of humankind's victory over disability, disease and death.

To read the formal remarks of speakers at the Lasker ceremony, as well as detailed information on this year's awardees, please refer to the Lasker website at: http://www.laskerfoundation.org/.