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Bioengineered bugs, drugs and contentious issues in patenting

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Bioengineered bugs, as is the scope of this journal, have great potential in various practical applications. A corollary to bringing useful products to the market is that such products need protection from copying by other people or businesses. Such government-sponsored protections are legally enforced through a patent, copyright or trademark/trade secret system commonly known as intellectual property rights. A condition for obtaining a patent is that the invention must not be disclosed to public either through seminars, informal public disclosures or publications in journals, although in the United States, there is a one year grace period that is allowed to obtain a patent after public disclosure. This article describes my personal experience in obtaining a patent in 1980 on a genetically manipulated bacterium designed for oil spill cleanup. This patent application went through a series of court cases that finally ended up in the Supreme Court of the United States. I also mention a similar contentious legal issue that is on the horizon and that the readers of *Bioengineered Bugs* should be aware of. Finally, I have taken the opportunity to describe my current efforts to bring to the market some unique potential multi-disease-targeting candidate drugs from *Pseudomonas aeruginosa and gonococci/meningococci* that, if found non-toxic and efficacious in humans, will revolutionize the drug industry. To ensure their marketability, we are trying to develop a patent portfolio that will ensure that they will be legally protected and such protections will be broad-based and enforceable.

Key words: anticancer drugs, business methods, drug promiscuity, HIV/AIDS, life form, malaria, patenting, *Pseudomonas aeruginosa*

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Introduction

Bioengineered bugs, more formally known as genetically engineered microorganisms, are products of genetic manipulation of microorganisms to improve their performance, whether it is production of a valuable chemical, enhanced yields of vitamins, amino acids or alcohol, production of antibiotics or other disease-fighting agents or simply enhanced immune development or stimulation through colonization of human guts. Thus engineering of bugs involves practical applications in biomedical, chemical, food/agribusiness as well as bioremediation and environmental restoration arenas. The new journal *Bioengineered Bugs* will address all these areas and more. If bioengineered bugs are designed to solve practical problems, they need to enter the global market not only as a commodity but also to bring economic prosperity to the developers and the nation. This means protection of the newly developed improved bugs from unfair copying and competition. While most academic researchers are adept at scholarly research and development, and eager to publish their findings in reputed journals, such as *Bioengineered Bugs,* they need to be cautious about losing their proprietary position in bringing the results of their research and invention to the market place. To be a commodity, a scholarly academic or industrial invention must be protected through a system called intellectual property rights (IPR). For scientific and technological innovations, IPR usually means patenting. Without a patent, very few scientific products, machines, processes or combinations thereof, can reach the market place because they will be

copied and the inventor will have no particular advantage in developing it. In this article, because of my past experience and the importance of creating and protecting intellectual property (IP) generated by scientific research through the legal system, I dwell at length on my involvement and the complexities associated with the patenting process[1] and some of my current research requiring patent protection.[2]

Patenting Life Forms

I obtained my Ph.D. degree in Biochemistry from the University of Calcutta, India, in 1965 and joined the laboratory of Dr. I.C. Gunsalus at the University of Illinois at Urbana in the same year as a postdoctoral fellow. In Urbana, my research involved an investigation of the genetics of nutritional versatility in the bacteria of the genus Pseudomonas[3] and the role of plasmids involved in the process.[4] During my postdoctoral years in Urbana, I learned that many degradative pathways for complex organic compounds, including hydrocarbons such as octane, decane, xylene, naphthalene, etc., are encoded by plasmids in pseudomonads such as *P. putida*.[3,4] Because of the transmissibility of many of these plasmids, they can be transferred from one strain to another, conferring on the recipient cells the ability to digest the hydrocarbons or even synthetic chlorinated compounds, as was shown later.[5,6]

With the new concepts of catabolic plasmids specifying degradation of hydrocarbons and other organic compounds fresh in my mind, I joined the Corporate Research & Development Center of General Electric Company (GE) in Schenectady, New York, in 1971 as a staff microbiologist. My assignment at GE was to develop a process to convert cow manure to cattle feed. In the southwestern part of the United States, there were large feed lots with 50,000 or more cattle that generated significant amounts of manure. Normally the manures were used as fertilizers or landfills but by early seventies, the United States Environmental Protection Agency banned the use of cow manure as fertilizers or landfills for the fear of contaminating crops or vegetables with pathogenic bacteria often present in manure. My GE colleague W. Dexter Bellamy and I developed a process using thermophilic actinomycetes that could digest the ligno-cellulosic component of the manure and grow at its expense, thus converting much of the manure to a protein (bacteria)-rich product that could be used as a cattle feed. However, I missed my work on the catabolic plasmids and their genetic divergence, evolution and expression. Because the GE Research & Development Center was an intellectually stimulating place that allowed different types of research and because enhanced hydrocarbon degradation through natural plasmid transfer allowed construction of multi-plasmid organisms with enhanced appetite for crude oil consumption[7] and therefore possible use in oil spill cleanup, I started to work on the construction of such a genetically improved pseudomonad at my spare time at GE. It is important to point out at this time for the benefit of my academic colleagues that while it is useful and indeed highly desirable to conduct cutting-edge research for practical problem solving and publishing the results in scientific journals, it is equally important to create intellectual property (IP) out of such research, if the results are ever going to find application in the real world. For most microbiological/biochemical processes or products, such generation of IP is accomplished through patenting. Consequently, before I published my observations on the construction and use of the multi-plasmid microorganisms in potential oil spill cleanup, GE filed a patent application to the United States Patent & Trademark Office (US PTO) on my behalf in 1972 to claim both the process of construction of the multi-plasmid microorganisms and the bioengineered bacterium itself.

The patent laws in the United States date back to 1793 to cover, in the words of Thomas Jefferson, one of the framers of the US Constitution, 'any new and useful art, machine, manufacture, or composition of matter, or any new or useful improvement thereof.' In an effort to upgrade the patent laws, the US Congress in 1952 replaced the word 'art' with the word 'process.' However, even as early as in 1889, the US Commissioner of Patents rejected a patent application on a fiber from the needles of pine trees, asserting that they are products of nature, lacked human intervention, and therefore remained non-patentable.

In 1973, the patent examiner of the PTO allowed the process patent for constructing the multi-plasmid organism but rejected the product patent on the organism itself, using the 1889 doctrine of the non-patentability of products of nature. In 1974, GE appealed this decision of the patent examiner to the PTO Board of Appeals, pointing out that the multi-plasmid organism was very different from its natural counterparts and was therefore not a product of nature. In 1976, the PTO Board conceded that the multi-plasmid bacterium was not a product of nature but rejected its patentability on the ground of its being alive, the so-called genetically engineered life form.

Court Cases on a Bioengineered Bug

One of the trickiest considerations in patenting an invention is to determine if the invention falls under the patentable subject matters as defined by the patent laws in the US Constitution. Is an invention, that satisfies all other statutory criteria, non-patentable simply because it is alive? There was nothing in the patent laws that addressed this question. Consequently, GE appealed to the Court of Customs and Patent Appeals (CCPA), a federal court, to overturn the decision of the PTO Board of Appeals. Indeed, in early 1978, the CCPA ruled three to two in our favor, contending that the bacterium carried out the hydrocarbon degradation through a series of chemical reactions, just as any chemical reagents and catalysts, and there was nothing that stood in the way of its patentability simply because it was alive.

In April, 1978, the Solicitor General of the United States appealed to the US Supreme Court to rule on the patentability of life forms. Initially, the Supreme Court sent the case back to the CCPA to reconsider in light of another case known as *Parker v. Flook*, but the CCPA reaffirmed its decision on the patentability of the multi-plasmid bacterium, this time by a four to one decision using the same arguments. An excellent account of the details

of this case is given by the Caltech historian Daniel J. Kevles.[8]

Having no other recourse, the Solicitor General petitioned the Supreme Court again in July, 1979, for review and in October of the same year, the review was granted. Many *amicus curiae* briefs were presented to the Court, primarily in support but also in opposition to the patentability of the bioengineered bug. While the supporters argued that the criteria for patentability should not be limited solely because the invention involves a life form so long as it meets all the statutory requirements of patentability (see also later), the opposition argued that life is a vital function that cannot just be an arrangements of chemicals, that granting patent to a 'soulless, mindless, lowly form of life' can lead to patenting of higher forms of life later on, perhaps including human beings, and that such an important decision in absence of a law should belong to the legislative body, the US Congress, requiring a new law, rather than made by the Court.

On June 16, 1980, eight years after the filing of my patent application, the Supreme Court ruled, by five to four, the eligibility of the multi-plasmid organism to a patent. The majority of the Justices cited the vision of Thomas Jefferson that 'ingenuity should receive a liberal encouragement', and declaring 'anything under the sun that is made by man' is patent eligible so long as it meets the statutory requirement of patentability. The minority Justices argued that patenting life form was not a Congressional intent, as the US Congress passed separate laws in 1930 known as the Plant Patent Act for asexually-reproduced plants and a Plant Variety Protection Act in 1970 to recognize the breeder's rights for protecting plant varieties for sexually reproduced plants. The minority argued that such specific Acts of Congress precluded other forms of life to be given patent protection and requires a new law. I have written many articles on my experience with the Supreme Court case known as *Diamond v. Chakrabarty* (447 US 303, 1980) where Sidney Diamond was the Commissioner of Patents.[9-11] I have also discussed the many aftermaths of the Supreme Court decision leading to the patenting of plants, animals, human cells including stem cells, genes, genetic mutations and a patent application on the construction of hybrid human-non-human animals, as well as many patent infringement and related cases.[1,12,13] There are some interesting differences between the US patent laws and the European patent laws that have also been emphasized, including a public order or morality clause in the European patent laws that is absent in the US patent laws.[1]

What's on the Horizon?

The *Diamond v. Chakrabarty* US Supreme Court decision in 1980 is believed to have helped the US biotechnology industry tremendously by allowing innovations to take roots in patenting and bringing to the market developments including regenerative medicines, antibodies, genetically-engineered microorganisms, animals, genes, cells, etc. As discussed above, the main question in this case was the eligibility of patenting of life forms under the US patent laws. No law can address the nature of all the potential innovations of the future and the US patent laws, as originally framed in the US Constitution in 1793 with subsequent amendments under Title 35, Section 101 (35 USC. section 101) state 'whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.' This simple statement of patent eligibility, however, has confronted the courts, and occasionally the US Congress, on the intricacies of various developments,[1] as exemplified by the question of patentability of genetically-engineered life forms as opposed to the products of nature where no human intervention takes place. Thus, as we will see hereunder, the Supreme Court decision on 'anything under the sun that is made by man' is patent eligible in the United States had a caveat, as elaborated in another case *Diamond v. Diehr*, 450 US 175, 182, 185, 1981, that 'laws of nature, natural phenomena and abstract ideas' are not patentable under US laws.

Are Business Methods, Information Technology and/or Internet Commerce Eligible for Patenting?

As foreseen by Thomas Jefferson, James Madison and other framers of the US Constitution, patent protection encourages innovation and should get liberal governmental support. However, the framers of the US Constitution, and the US Congress, could not foresee all the complexities of future innovations. Thus the patent laws have been mired with difficult issues, particularly involving processes and methods in internet commerce that is today a business of several trillion dollars. An emerging interesting case on the horizon that the US Supreme Court decided to act on is a patent eligibility case involving a business method. The patent application was filed by Bernard L. Bilski and Rand A. Warsaw in April, 1997, on how to hedge commodity-associated risks, particularly for energy commodities such as natural gas or electricity, and was entitled 'Energy Risk Management Method.' The claimed method of hedging the risks associated with the consumption of a commodity such as natural gas used for home heating during winter months involves both natural gas suppliers and consumers. The claims outline how the suppliers and the consumers can agree on a fixed price throughout the year or winter months, even if the severity of winter affecting the natural gas price varies significantly. The application was rejected by the patent examiner under 35 USC. section 101 as being an extension of an abstract idea and a mathematical deduction that remains patent ineligible. The applicants appealed this rejection to the PTO Board of Patent Appeals and Interferences (BPAI) under section 141 of 35 USC. This appeal was rejected by an expanded panel of the BPAI in September 2006, again on grounds of the claims representing abstract ideas without involvement of any machine or transformation of one material to another, a condition of the eligibility of a process patent that was thought to be the clue for patentability as noted by an earlier Supreme Court decision. The patent applicants appealed this BPAI rejection to the Court of Appeals for the Federal Circuit (CAFC) that came

to be known as the *Bilski* case. This case was considered by a three-judge panel of the CAFC in October 2007. Considering the importance of the broader question of patent eligibility of business methods and processes, the CAFC decided to hear the case *en banc*, meaning all the 12 judges of the CAFC participated in the decision making process.

The question of patenting business methods, particularly those that involve no machines or physical/chemical transformation but sheer mental exercises, is murky. Such methods are widely used by financial services, management consulting and other business groups. Consequently, such groups have great interest in developing proprietary positions through patenting business methods and processes. The *en banc* participation by the CAFC is a reflection of this widespread interest in patenting complex business methods and if such methods are patent eligible under the US laws. This question is somewhat similar to the question of patent eligibility of life forms in the seventies as decided by a predecessor of the CAFC called Court of Custom and Patent Appeals (CCPA) as mentioned earlier. Thirty-eight *amicus* briefs (*amicus curiae*) were submitted by various interested parties to the CAFC, either to support or to oppose the patent eligibility of the *Bilski* application. First oral arguments were held in May 2008. In October, 2008, in a nine to three divided decision, the majority in the *en banc* CAFC held that *Bilski* claims were not patent eligible under section 101 since the claims constituted mental exercises, somewhat similar to abstract ideas that have been considered non-patentable by the previous Supreme Court decisions. To be patent eligible under section 101 of the US Constitution, the CAFC ruled that an application for a process patent must satisfy two criteria. The process must involve a machine or it must involve transformation of an article to a different state or thing. This majority decision of the CAFC was primarily based on a 1981 Supreme Court decision known as *Diamond v. Diehr*, 450 US 175, where the involvement of the transformation of an article, in absence of that of a machine, was described as the clue to patent eligibility. The *Diehr* application involved claims to a process for producing cured synthetic rubber products, taking temperature readings during curing and making use of mathematical equations to calculate optimum curing time. Even though mathematical calculations are not patent eligible by themselves, the Supreme Court allowed this process patent for curing rubber using that equation, ruling that a patent on the equation itself, similar to laws of nature, natural phenomena or abstract ideas, cannot be granted but the useful application of the mathematical formula to a process such as optimum time for curing rubber is patent eligible.

The rejection of the patent eligibility of the *Bilski* claims by the CAFC, interestingly, appears to be a reversal of earlier decisions by the same court, known as *Alappatt* 1994, *State Street Bank* 1998 and *AT&T* 1999 cases, where the court wanted a process-related innovation to produce 'useful, concrete and tangible' results for patent eligibility, rather than a highly restricted machine/transformation test. Two circuit (CAFC) judges, judge Pauline Newman and judge Randall Rader, highly respected and well-known experts in biotechnology and pharmaceutical related patent infringement and liability cases, dissented. They pointed out that such restrictions go beyond the statutes and will have drastic negative effects on the spirit of innovation, including medical diagnostics and many other processes involving modern day intellectual activities, and in fact go against the Congressional intent of subject matter eligibility for patenting. Whether *Bilski* should be granted a patent or not should be decided on various criteria such as novelty (section 102), non-obviousness (section 103), written description and enablement (section 112), etc., or if the claims fall under non-patentable subject matters such as abstract ideas. In general, the questions for business methods as patentable subject matters are daunting, as exemplified by data processing system for management of financial services or automatic routing of telephone calls. Diagnostic process patents in medical biotechnology have also remained somewhat in limbo. There is a case *Laboratory Corporation of America Holdings v. Metabolite Laboratories*, 548 US 124 (2006), involving the measurement of homocysteine levels in body fluids to correlate with cobalamin or folate deficiency. The US Supreme Court initially granted Metabolite Laboratories's application for review on the question of whether a well-known scientific fact in the medical literature can be eligible for a patent, but later declined to review it. Thus the acceptance on June 1, 2009, of the *Bilski* case by the Supreme Court for review is expected to provide guidance for determining patent eligibility of many business-related processes that remain dormant in the courts. Indeed, in *re Bilski*, the CAFC did not rule out that the strict machine/transformation interpretation of business method/process patent eligibility is here to stay. The CAFC did recognize the unpredictable nature of future innovations, particularly in internet commerce involving trillions of US dollars, and kept the door open for a change of the machine/transformation requirement of process patent eligibility. It would be interesting to see how the Supreme Court rules on this issue. As for my part, two highly respected Law Professors, Professor Richard Epstein of the University of Chicago and Professor F. Scott Kieff of the George Washington University of Washington, DC, filed, graciously and *pro bono*, an amicus brief to the Supreme Court on my behalf supporting the patent eligibility of the *Bilski* application, but not necessarily its issuance unless it satisfies all the other patenting criteria mentioned earlier.

Bioengineered Bugs as Sources of Drugs

In the above two sections, I have discussed the complexities of patent eligibility criteria, particularly as they relate to life forms or business method developments. The idea is to sensitize the readership of Bioengineered Bugs to the need for generating and protecting their research results that are likely to have practical applications. Both biotechnological products and processes should meet patent eligibility criteria that the readers need to know. In this section, I would briefly mention some of my current research and how I am trying to create intellectual property to bring the results of my research to the market place.

My current research, similar to my previous research on Pseudomonas, involves the opportunistic pathogenic bacterium *Pseudomonas aeruginosa*. A great deal is known about how *P. aeruginosa* infects debilitated, immuno-compromised or other vulnerable patients such as those suffering from cystic fibrosis, to cause chronic infections.[14] *Pseudomonas aeruginosa* is well-known to harbor pathogenicity islands that encode various virulence factors that are secreted through the type III secretion system to take over the host cells. On taking over, *P. aeruginosa* forms biofilms on epithelial cell surfaces for a slow mode of growth, leading to long term residence and resistance development against antibiotics or immune attacks.[14] Biofilm formation in cystic fibrosis patients' lung epithelial cells by infecting *P. aeruginosa* has been demonstrated, where the extremely slow growth of the bacteria produces no immediate toxicity problem (except clogging the lungs with the mucus), thereby assuring long-term co-existence with the host. An interesting question in this context is if the resident bacteria would consider their host as their niche and be protective of their turf from invasion by other disease agents. Such agents can either compete with the resident bacteria for the same niche, or simply kill the host in which case the bacteria lose their sanctuary.[15] Such disease agents could either be internal, such as cancer, or external such as a virus, HIV-1 for example, or a parasite such as the malarial parasite *Plasmodium falciparum* or the toxoplasmosis-causing parasite *Toxoplasma gondii*. Since *P. aeruginosa* has a relatively small genome size of about 6 megabases while there are many viruses, parasites or even cancers, it is apparent that if *P. aeruginosa* indeed needs to defend its turf by producing a weapon, or a limited number of weapons, directed against a multitude of invading enemies, the weapon or weapons must be exquisitely designed to target and be simultaneously effective against many of these diverse enemies.

We have recently reported that *P. aeruginosa* secretes a periplasmic protein azurin, as well as CpG-rich extrachromosomal DNA, on exposure to cancer cells.[16] It has been well-known that many bacteria, such as Salmonella, Clostridia and others, can allow cancer regression in cancer bearing animals or even humans. Indeed, *Mycobacterium bovis* BCG is widely used in the treatment of bladder cancer.[16] We previously demonstrated that azurin, as well as an azurin-like protein called Laz from *gonococci/meningococci*, had strong anticancer activity against such human cancers as melanoma, breast cancer and glioblastoma,[17-19] giving these proteins the status of bacterial weapons against internal invaders. Such anticancer activity could be demonstrated not only in vitro but also in vivo in nude mice where azurin was demonstrated to allow melanoma regression by 59%[17] and breast cancer regression by 85%[18] without showing any detectable toxicity symptoms in such mice.

An interesting feature of azurin's anticancer activity is its promiscuity in targeting multiple pathways by which cancer cells grow.[2] Cancer cells are normally resistant to undergoing apoptotic cell death due to mutational inactivation or epigenetic silencing of the expression of genes encoding tumor suppressor proteins such as p53. Azurin promotes apoptotic cell death in cancer cells through complex formation and stabilization of p53.[17,18] Azurin also inhibits angiogenesis and receptor tyrosine kinase-mediated cell signaling that promotes cancer growth.[20] FDA-approved and currently used anticancer drugs, particularly rationally designed drugs, have a single or limited number of targets, such as one or two receptor tyrosine kinases.[2] The cancer cells respond to such drugs by quickly changing the target, thereby becoming drug resistant. Cardiotoxicity or other forms of toxicity are also often problematic in prolonged use of such drugs because the rationally designed tyrosine kinase inhibitors inhibit other physiologically important tyrosine kinases, of which there are 518 in the human kinome, as well.[2] It would be interesting to see if azurin will be less susceptible to resistance development because of its multi-targeting nature as well as its mode of inhibition of the tyrosine kinase (preventing ligand binding rather than occupying the ATP-binding pocket).[2] It is to be noted that azurin can also prevent cancer emergence as judged by its ability to inhibit (70%) precancerous lesion formation in growing normal mammary cells, when such cells are exposed to a carcinogen such as 7,12-dimethyl-benzanthracene (DMBA), in murine mammary organ culture (MMOC) assays.[21] The anticancer (cancer-killing) activity and the cancer preventive vaccine-like activity of azurin can potentially make azurin an excellent cancer-fighting drug of the future.[2]

A second unique feature of azurin is its promiscuity in attacking multiple diverse disease agents such as the AIDS-causing virus HIV-1, the malarial parasite *P. falciparum* or the toxoplasmosis-causing parasite *T. gondii*.[22,23] The ability of azurin to prevent invasion by these attackers of the human body has been shown to be due to azurin's unique structural features similar to the immunoglobulin variable domains. Azurin is a member of a family of copper-containing redox proteins known as cupredoxins. These two groups of proteins, cupredoxins and immunoglobulins, share a common beta-sandwich packing structure.[14] Such structural similarity endows on azurin the ability to bind many important antigens present on the surface of parasites, viruses or even cancers. For example, azurin enters preferentially to cancer cells[24] and binds the tumor suppressor protein p53 with a K_d value of 33.0 nM, leading to its stabilization and induction of apoptosis in cancer cells. Similarly, azurin demonstrates structural similarity with the ligand ephrinB2 and binds the receptor tyrosine kinase EphB2 with a higher affinity (Kd value 6.0 nM) than ephrinB2 (Kd value 30.0 nM), interfering in EphB2-ephrinB2 signaling that promotes cancer growth.[2,14,20] With regard to inhibition of parasite invasion of host cells, azurin binds the merozoite surface protein MSP-1 of *P. falciparum* with a Kd value of 32.2 nM[22] and the surface antigen SAG1 of *T. gondii* with a Kd value of 12.8 nM.[23] Both MSP-1 and SAG1 are important surface components of the parasites for their invasion of hosts and growth inside such hosts. By binding such important surface components, azurin interferes in their invasion. Another interesting aspect of azurin's growth inhibitory action is towards the AIDS-causing virus HIV-1. Both azurin and the azurin-like protein Laz have strong inhibitory action not only towards *T. gondii*[23] and *P. falciparum* but

Table 1. Issued US patents covering diverse multi-disease-targeting activities of azurin, Laz and peptides derived therefrom

U.S. patent number	Title	Inventors	Issue date
7, 084, 105	Cytotoxic factors for modulating cell death	Chakrabarty AM; Das Gupta TK; Punj V; Zaborina O	August 1, 2006
7, 301, 010	Compositions and methods for treating HIV infection with cupredoxin and cytochrome *c*	Chakrabarty A; Das Gupta T; Yamada T; Chaudhari A; Fialho A; Hong CS	November 27, 2007
7, 338, 766	Compositions and methods for treating malaria with cupredoxin and cytochrome	Chakrabarty A; Das Gupta T; Yamada T; Chaudhari A; Fialho A; Hong CS	March 4, 2008
7, 381, 701	Compositions and methods for treating conditions related to ephrin signaling with cupredoxins	Chakrabarty A; Das Gupta T; Yamada T; Chaudhari A; Fialho A; Zhu Y	June 3, 2008
7, 491, 394	Cytotoxic factors for modulating cell death	Chakrabarty AM; Das Gupta TK; Punj V; Zaborina O; Hiraoka Y; Yamada T	February 17, 2009
7, 511, 117	Compositions and methods for treating HIV infection with cupredoxin and cytochrome *c*	Chakrabarty A; Das Gupta T; Yamada T; Chaudhari A; Fialho A; Hong CS	March 31, 2009
7, 556, 810	Compositions and methods to control angiogenesis with cupredoxins	Mehta RR; Taylor BN; Yamada T; Beattie CW; Das Gupta TK; Chakrabarty AM	July 7, 2009

also towards HIV-1.[22] Because HIV-1 is highly mutable and acquires resistance to drugs that target and inhibit its essential replication/maturation machinery such as reverse transcriptase or protease, a combination (cocktail) of such anti-retroviral drugs is used to treat AIDS. While such drug combination reduces HIV-1's drug resistance, it adds to the cost of the treatment and the potential development of multi-drug resistance. An ideal anti-retroviral drug should not only target the HIV-1 entry or replication machinery, but also host components that are important in HIV-1 transport from the mucosal surface to the lymphatic T cells and HIV-1's entry to the T cells. It is thus interesting to note that azurin not only targets HIV-1 gp120, an important envelope protein that binds CD4, with a Kd value of 8.9 nM, but also host proteins such as ICAM-3, important for HIV-1 entry, with a Kd value of 20.2 nM and DC-SIGN, an important dendritic cell surface protein that binds gp120 and allows HIV-1 transport to the T cells. Azurin binds DC-SIGN with a Kd value of 0.83 nM.[22] Since HIV-1 cannot mutate to change the host proteins, such binding of azurin with host proteins is likely not only to interfere in HIV-1 transport and entry but also to reduce HIV-1's ability to become resistant to azurin, if azurin is proven efficacious in AIDS treatment or infection.

Since azurin is a protein, there is concern that its stability in blood will be low. Although azurin is quite non-immunogenic, its stability in blood can be enhanced by conjugation with polyethylene glycol (PEG), a known protein stabilizer. However, synthetic peptides are considered more appropriate for use as drugs, as opposed to protein biologics. It is interesting to note in this context that a 28 amino acid peptide derived from azurin, termed P28 comprising of azurin amino acid residues 50–77, has not only entry specificity to cancer cells, but also anticancer, including anti-angiogenic, activity[24,25] as well as cancer preventive properties.[21] P28 has been shown to have no toxicity in animals and is undergoing phase I human clinical trial in the United States as an anticancer agent (www.cdgti.com). Another candidate peptide for use both as a potential anticancer and anti-HIV/AIDS agent is the 26 amino acid peptide P26 comprising azurin amino acid residues 88–113. A part of this peptide, residues 96–113, has structural similarity to the G-H loop region of ephrinB2 that is important for its binding with the receptor EphB2. Indeed, a GST-azurin 88–113 fusion protein has been shown to have anticancer activity, as compared to the control GST which has none.[2,20] The larger azurin 88–113 peptide (P26) can strongly bind DC-SIGN, the dendritic cell surface protein with a Kd value of about 5.0 nM. It remains to be seen if such binding between P26 and DC-SIGN might prevent the dendritic cells to transport the HIV-1 virus to the T cells, thereby preventing HIV-1 infection in susceptible people. The ability of azurin/Laz as well as the chemically synthesized P26 peptide to suppress HIV-1 invasion of T cells and growth, as well as potentially interfere in the HIV-1 infection, thereby acting more like a vaccine where none exists, can be exciting developments for the future to treat AIDS patients as well as people vulnerable to HIV infection and AIDS development. Since AIDS patients often have sarcomas or get co-infected with *P. falciparum*, *T. gondii* or other pathogens because of lowered immunity, and given the promiscuity of azurin to attack cancers, HIV-1 and parasites, it will be of great interest to see if a single candidate drug such as azurin, that can be mass produced cheaply, can be used in the treatment of AIDS patients co-infected with cancers, parasites or other pathogens.

Since we are quite serious about bringing azurin, Laz or the chemically synthesized peptides to the bedside and the market place, we have set up a start-up company and filed many patent applications to cover our findings. Seven US patents, belonging to the University of Illinois, have already been issued (**Table 1**) and many others, including international patents, are in the process of being issued. A list of the issued US patents and their contents and related publications as well as the status of the P28 clinical trials are given at www.cdgti.com web site. If azurin/Laz or any of the peptides derived from them are found to be non-toxic and efficacious in humans against cancer, HIV/AIDS, malaria or combinations thereof, it would be of great interest to look for other pathogenic bacteria that cause chronic infections in humans, and thereby having a long term niche in the body, as the source of many such candidate drugs of the future.

It is important in this context to realize that azurin is not the only candidate drug produced by *P. aeruginosa*. Another protein, termed Pa-CARD, which is a truncated version (17 kDa) of the enzyme arginine deiminase (ADI, 46 kDa) from *P. aeruginosa*, has been shown to be active against leukemia and other types of cancer.[26,27] Pa-CARD harbors a caspase recruitment domain (CARD) that is normally present in eukaryotic, primarily mammalian, cells. Microarray experiments suggest that Pa-CARD modulates NFκB signaling pathway genes to exert its anticancer activity.[27] The anticancer activity of Pa-CARD is mediated through cell cycle arrest at the G_2/M phase involving Wee1 protein stabilization and the depletion of phosphorylated AKT-Ser-473, the active form of a serine/threonine kinase that is often dysregulated in many cancer types.[26] Thus it would be interesting to explore if other bioengineered bugs would prove to be a similar source of many such drugs of the future. Similar to many antibiotics produced by many bacteria, azurin or Laz type of proteins with high multi-disease-targeting ability might be produced by many other bacteria or fungi/protozoa, making bioengineered bugs a valuable resource to humankind and spawning a new type of drug industry.[2,14,15]

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