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Short Communication

Targeted Therapies: Cellular and Molecular. Point of View

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Several decades ago, we at the University of Minnesota, initiated studies to explore cell signaling mechanisms, that modulate the physiology/pathology, functions/dysfunctions, at cellular, tissue, and organ levels. We used a variety of molecules, to study the process at the ultrastructure level, as well as at biochemical, and molecular levels [1,2]. Our department, (Laboratory Medicine and Pathology) also organized, one of a kind course on 'Mechanisms of Diseases." In our exploratory studies, we used several molecules such as Vincristine, Cytochalasin, Colchicine, Taxol, Calmodulin, Amiloride, and calcium ionophore, A23187 [3-12]. Little did we know at that time, that some of these molecules, will become major anti-cancer drugs. Drug discovery and development takes a fairly long process. For instance, Paclitaxel (Taxol), the most well-known natural-source cancer drug was collected by USDA in 1962, yet it took decades until 1977 for the National Cancer Institute (NCI), to confirm antitumor activity in the mouse melanoma model. It took another decade for NCI, to begin phase one clinical trial of Taxol, against a number of cancer types. In December 1992, the FDA approved Taxol for the treatment of ovarian cancer. In 1994, the FDA approved Taxol for use against breast cancer. Vinca alkaloids are administered with cyclophosphamide, doxorubicin, prednisone, methotrexate, procarbazine, and dacarbazine. The limitation of vincristine therapy is its neurotoxicity [13,14]. In the early 90s, most drugs used in cancer treatment worked by killing cells, that were in the process of replicating their DNA, and dividing to form new cells.

Targeted therapies, on the other hand, work by modulating or influencing the process that controls growth and spread of malignant cells. These therapies are aimed at inhibiting some specific, essential functions of cancer growth and proliferation, such as growth signal inhibitors, angiogenesis inhibitors, energy-depleting drugs, and apoptosis-inducing drugs. In the early 80s, researchers recognized that growth factors and other modulators, elicited their response through oncogenes. The earliest targeted therapies for growth factor signaling included trastuzumab (Herceptin), gefitinib (Iressa), imatinib (Gleevec), and Cetuximab (Erbitux). Angiogenesis provides tumors, with the extra blood supply needed for proliferation and growth. Despite the concept being known in the 70s, it was not till 2004, that the first angiogenesis inhibitor (Avastatin) was approved. Apoptosis is the natural process of aging, which leads to the damage of DNA, initiates the repair process, and in case of excess damage, results in the death of the cells. Many of the current therapies rely on this mechanism, including radiation and chemotherapies. Whereas targeted toxins are a class by themselves, which have tumor-selective ligands coupled to polypeptide toxins. This class of bio-engineered drugs kill malignant cells by inactivating cytosolic protein synthesis and inducing apoptosis. The targeting protein may be an antibody or antibody fragment.

These immunotoxins direct the toxic molecules to a cell surface receptor, then these molecules are internalized, once in the cytosol, they induce apoptosis by inactivating protein synthesis. These toxins are so potent, that as few as a single molecule can kill a cell. The toxins used for targeted cancer therapies include, diphtheria toxin (DT), pseudomonas endotoxin toxin (PE), ricin from castor seeds, *Gelonium multiflorum* (GEL), and Poke weed antiviral protein (PAP) from plant sources. Impressive results have been obtained with ONTAK, which is composed of catalytic and transmembrane domains of DT fused to human IL-2. It was approved by the US/FDA in 1999 for sale, as targeted cancer therapy. It was eventually withdrawn from the market due to production issues. Although several immunotoxins are in the development and testing phase, the use of these antibody-coupled toxic molecules has some drawbacks. They are seen as 'foreign' by the innate immune system. Although just two drugs have been approved for therapeutic purposes, researchers are trying to modify the antibody and toxin domains in novel ways, to reduce the immunogenicity of these toxins and associated antibodies [15].

Sensitization of malignant cells to therapies

Hyperthermia uses heat to raise the tissue temperature, to as high as 113^0F to help kill cancer cells with little damage to normal cells. The hyperthermia treatment includes regional hyperthermia, whole-body hyperthermia, and hyperthermic intraperitoneal chemotherapy. Currently, the most widely used method for hyperthermia in clinical practice is, the use of a 9MHz or 13.56MHz radiofrequency heating device, applied to the surface of the body directly above the tumor. Although hyperthermia is considered a safe and effective way, to potentiate the effect of anticancer therapies, it is still difficult to aggregate the heat effect only in cancer tissues. Multicenter clinical studies using this kind of combination, are needed to provide convincing evidence [16]. In the early 90s, studies at the University of Minnesota demonstrated, thermosensitization of tumor cells by increasing intracellular acidity with amiloride and its analogs [17]. According to the authors of this study, both amiloride and its analog, significantly enhanced the thermal effect on tumors *in vivo* as judged by tumor growth delay, and also *in vitro-in vivo* assay for oncogenic cells.

In recent years, there is considerable interest in developing therapies for the treatment of so-called incurable cancers, such as pancreatic cancer, glioblastomas, and melanomas. In our earlier ultrastructure studies at the University of Minnesota, we used nano gold particles coupled with fibrinogen to follow the receptor-mediated transport of fibrinogen in platelets [18]. Nanotechnology has become a trending area and gold nanoparticles have found some very useful medical applications [19]. Rao Papineni and associates have reported a unique pancreatic cancer treatment platform, where X-ray excited Targeted-Gold nanoparticles (T-GNPs) trigger and enhance drug action (I-RaGAZ, Integrated Radiation Combined with T-GNP and 12-Azido-2deoxy-D glucose (2-AZ-2-DG) [20]. These authors have studied the early time point gene expression in SK-N -AS and SK-N-DZ cells, in response to radiation as cell-specific 'strategies' to recover from the radiation damage [21]. Molecularly targeted inhibition of the different components of the nuclear factor kB kinase (IKK)-nuclear factor kB (NFkB) pathway, is widely explored for the treatment of cancer alone or in combination with other cancer therapies [22].

Therapies awaiting clinical validation

An important feature of rapidly proliferating cancer cells is their ability to modulate their energy metabolism, to sustain the production of ATP and macromolecules needed for growth, proliferation, and survival. We have already briefly discussed the growth factors and neovascularization. In this section, we will briefly discuss glycolysis and lipidomic pathways for tumor proliferation, and some therapeutic approaches to interfere with these mechanisms. According to Prof Pederson and associates, at the Johns Hopkins University School of Medicine, a common feature of most advanced cancers is their capacity, to metabolize glucose to lactic acid. In a highly glycolytic hepatocellular carcinoma animal model, these researchers used alkylating agent 3-bromopyruvate (3BP), and eradicated advanced cancers without apparent toxicity [23]. In a seminal paper on this topic Prof. Pederson writes, "despite more than 75 years of research by some of the greatest scientists in the world to conquer cancer, the clear winner still is cancer". It has not changed even after a century of intense research on this topic. In this article, they mention the uniqueness of malignant cells, -the overexpression of a mitochondrial-bound form of hexokinase Type II, and how this knowledge can lead to improved therapies [24]. In January 2013, Nobel laureate James D Watson paid homage to 3BP and, to the work of Pederson and Ko on their findings.

3-bromopyruvate, the powerful dual inhibitor of hexokinase, as well as oxidative phosphorylation, kills highly dangerous hepatocellular carcinoma cells, more than 10 times faster than the more resilient normal liver cells, and therefore, has the advantage to truly cure, an otherwise highly incurable cancer. According to these researchers, 3BP enters the cell through the highly expressed pyruvate receptors, similar to the "Trojan Horse" hypothesis, sneaks in through the receptors that lactate uses, and selectively depletes ATP from cancer cells. In our effort to understand why such a viable approach has remained 'orphan', we tried to promote limited clinical studies on this drug in India. Since this drug is not approved for use by any regulatory agencies, one has to start from phase 1 trials. PreScience Laboratory, a U. S company has received approval for a phase 1 study from the US/FDA. Gershwin the founder says, the trial has yet to start because the company needs partners to finance the project. Furthermore, there appears to be legal disputes about its use, going back to the original researchers at Johns Hopkins. We faced similar hurdles in India, to start a phase-1 studies on 3-bromopyruvate.

Yet another metabolic hallmark of cancer cells, seems to be lipidomic remodeling, which encompasses alterations in Fatty Acid (FA) transport, de novo lipogenesis, storage of lipid droplets, and β-oxidation to generate ATP [25]. According to these researchers, fatty acid translocase (FAT), fatty acid transport family (FATPs), and solute carrier proteins (SLC27), are expressed highly in tumors, especially on the cluster designate (CD)-36. Expression of excess CD36 indeed seems, to be a poor prognosis across several tumor lines, including breast, ovarian, gastric, and prostate cancers. Furthermore, tumor cells are characterized by high fatty acid supply and upregulation of lipogenesis [26-28]. Hoxha and associates from Albania, have reviewed the role of fatty acids in colorectal cancer progression [27]. They raise some very important questions, how does FA pathway influence the adenoma and carcinoma sequence and progression? How do FAs influence tumor progression, tumor resistance, and tumor metastasis? Professor Das and associates have reviewed the role of polyunsaturated fatty acids (PUFAs), on drug-sensitive and resistant tumor cells [28].

One of the well-known incurable cancer is gliomas, and usually affects the cerebral hemispheres. Several studies have shown that fatty acids such as -linolenic acid (GLA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), can selectively kill tumor cells without harming normal cells [29]. In a limited open, collaborative US/India clinical trial, Das, and associates demonstrated, that intratumoral administration of GLA, was found to be beneficial for the treatment of human gliomas. The duration of treatment in this limited study was only 7 days, and the dose administered was 7.5 to 15 ug/kg. Further large-scale double-blind randomized clinical trials are needed, to demonstrate the beneficial effects of PUFAs as anti-cancer therapeutics. This is also true for those studies, in which FAs are used for interfering with energy metabolism of tumors.

Emerging anti-cancer therapies

According to National Cancer Institute (NCI), USA, one of the most exciting developments in the fight against cancer is the advent of cell-based immunotherapy, a personalized treatment, that kills cancer using the patient's immune cells. Cellular therapies have been approved for people with certain blood cancers [30]. However, for treating solid tumors, it is still in the research and developmental stage. Chimeric antigen receptor (CAR) T cell therapy (CAR- T cell therapy), is an example of cell-based gene therapy. This kind of therapy combines the technologies of gene therapy and cell therapy. Since 2017, six CAR T-cell therapies (Kymirah, Yescarta, Tecartus, Breyanzi, Abecma, Carvkti) have been approved by the FDA. Now, these therapies have been widely available in the USA, and have become standard treatments for lymphomas. T-cells obtained from patient's own peripheral blood cells, are modified to express a receptor, that binds to the tumor antigen. An NCI team reported, that approximately 50% of those children who received CAR T-cell therapy, are still alive 5 years later without cancer coming back. All of the currently approved CAR T-cell therapies, rely on the disarmed virus, to deliver the genetic material into T cells to produce the CAR.

Currently, gene-editing technologies like TALON and CRISPR are used to produce CARs. Furthermore, studies are in progress for using nanotechnology and mRNA-based approaches, to develop CAR T-cells to be created inside the body. CAR T-cell has not been proven to be effective for the treatment of solid tumors. Studies are in progress, to develop tumor-infiltrating lymphocytes (TIL), which are a group of

intratumor lymphocytes for adoptive cellular therapy (ACT). The TIL-ACT process starts, with the isolation of the natural infiltrating lymphocytes from the tumor tissue, expanding them *in vitro* and, then infusing them with high doses of IL-2 into patients, to identify and kill tumors [31]. Researchers at the Center for Cellular and Immunotherapies, Perelman School of Medicine, University of Pennsylvania declared "two patients as 'cured' of leukemia, a decade after the innovative treatment, that has transformed blood cancer care. In this study, they followed the long-lasting effect of CD19-redirected chimeric antigen receptor (CAR) T cells, in two patients with chronic lymphocytic leukemia [32]. In a recent TIL trial, 168 patients with metastatic melanoma were randomly assigned to receive either TIL or the current standard treatment, immunotherapy with ipilimumab. Compared to those who were treated with drug, patients on TIL, had a 50% reduction in disease progression and death.

When I joined the department of pharmacology, at the University of Minnesota, as a post-doctoral fellow (NIH Fellowship) in 1971, the department was ranked one of the top ten research platforms in this field. It was well known for research on drug metabolizing liver enzymes,-the cytochrome P450. The field of pharmacology research has changed significantly in the last half-century. An important milestone in the history of drug development is the discovery of insulin [33]. In 1923 Banting and MacLeod were awarded Noble Prize for Physiology and Medicine. The discovery of insulin played a great role in the management of diabetes. Since this milestone discovery, pharmaceutical sciences have made lots of progress. Vertex Pharmaceuticals of Boston, MA, reported the results of a trial, in which infused cells grown from stem cells, behaved like the insulin-producing pancreatic cells, in the first-ever human studies of this kind [34]. The New York Times (November 27, 2021) published an article titled, "A cure for Type-1 Diabetes? For one Man, It Seems to Have Worked". The challenge these researchers faced was, to find out what sequences of chemical messages, would turn stem cells into insulin-secreting islet-like cells [35]. The discovery of another set of molecules, 'antibiotics' gave us the ability to control infectious diseases.

Then came the discovery of the structure of DNA, which contains the instructions needed for organisms to develop, survive, and replicate. The knowledge gained over the years on the workings of DNA, allowed us to reconstruct and reprogram a messenger RNA, to use as a therapeutic agent for the killer virus COVID-19, at the shortest possible time. Cell therapy that we have discussed earlier may be further enhanced by mRNA research and applications. The cells may be obtained from the patients, and subsequently modified *ex vivo* with mRNA, encoding the desired proteins, such as CARs, reprogramming, and trans differentiation factors [36]. For example, the BCL2 family of genes plays an important role in the apoptosis of different types of tumors. When it comes to the development of anticancer therapies, researchers have used cytotoxic agents which elicit therapeutic effects in part through the induction of apoptosis. However, more recently drugs have been developed to influence the genes responsible for apoptosis [37-39]. Since there is a lot of interest in the future of gene expression studies, it is essential to develop methods and screening techniques, to elucidate gene function to the discovery of prospective therapeutic targets. The CRISPR-Cas9 system has become a central tool in search, by enabling genetic screens to be easily performed at the genome scale [40].

Another emerging technology is to develop tumor-specific vaccines. Some researchers are testing tumor antigens, molecular markers that are few on normal cells, and plenty on tumor cells. The Lunch vaccine targets "neoantigens,' a potent type of antigen only found on tumor cells. These efforts are modeled on the messenger RNA (mRNA) vaccines, that were developed to fight the COVID-19 pandemic and could be extended to meet the ambitious goal set by President Joe Biden. "We are a long way from a general vaccine" to prevent cancer says, medical oncologist Shizuko Sei of the NCIs Division of Cancer Prevention". "But it could be in the distant future. It is a stepwise approach". In 1989 Oliver Finn of University of Pittsburgh discovered the first tumor-associated antigen; a version of MUCI, a sugar-laden cell-surface protein. Further studies by these researchers, have demonstrated that trigger B cells, to make antibodies. However, the immunologists at Penn Medicine's Abramson Cancer Center suggest, that the best approach is to inject genetic instructions for the antigen rather than the antigen itself, similar to the mRNA vaccines for COVID-19. The team of researchers from this Cancer Center, are testing a DNA-based vaccine targeting a different antigen that marks many tumors: hTERT.

Results of the hTERT trial, testing the safety of the vaccines in 93 patients in remission, after treatment for various cancers are encouraging. According to a Science Report (doi: 10.1126/science.abq3411, 2022), all but four patients made T cells that home in on hTERT and the vaccine was successfully warding off cancer. Among 34 people who had pancreatic cancer, 41% were still cancer free after 18 months. "The fact that mRNA vaccines have shown safety in billions of healthy people of all ages, makes mRNA a very good platform, for future RNA-based therapies". In a concept paper, the Advanced Agency for Health (ARPA-H) puts the goal this way: "Use mRNA vaccines to teach the immune system, to recognize 50 common genetic mutations that drive cancers, so that the body will wipe out cancer cells when they first arise". The use of the knowledge learned in the development of mRNA therapeutics, will be the basis for a list of potential projects for a reignited 'Cancer Moon Shot' and the new high-risk, high-reward research agency.

Noncommunicable disease like coronary artery disease and cancer, are the major killers of this century. Cardiovascular diseases (CVD), are the leading cause of death globally, taking an estimated 17.9 million lives each year. In 2006, Professor (Sir) Vijay K Kakkar, Director, Thrombosis Research Institute, UK, inaugurated Thrombosis Research Institute, in India. His specific goal was to develop vaccines for the prevention of coronary artery disease. His was a conventional approach to find a specific protein/proteins as biomarkers for CVDs, and then develop CVD-vaccines for those biomarkers. As we have learnt from experience, we can develop vaccines for metabolic diseases also, provided we understand basic mechanisms responsible for initiation and progression of these diseases. Once specific biomarkers are developed for various risk factors for metabolic diseases, it is possible to develop appropriate, effective interventions. There are infinite possibilities for developing targeted therapies for various metabolic diseases.

Over the past few years, we have seen rapid development and application of broad molecular testing of individuals, for early diagnosis of metabolic diseases as well as cancers, through analysis of DNA, RNA, genes that code for various signaling mechanisms, as well as for biomarkers for various diseases. The progress in this field is so rapid, that cost of whole genome sequencing (WGS) over the years, has come down from $300 million for the first one in the year 2000, to now, starting at $100. According to Eric Topol, Professor of Genomics, The Scrips Research Institute, Sand Diego, California, whole genome sequencing for sick newborn children, is now being performed in 83 children's hospitals in the US and Canada. The American Society of Clinical Oncology and the National Cancer Institute have taken lead in organizing large trials (TAPUR and NIC-Match), that have enrolled thousands of cancer patients within the past year. We sincerely hope that this trend will continue, and remarkable progress will be made in the field of personal medicine, precision medicine, and targeted therapies.

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