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Production of recombinant immunotherapeutics for anticancer treatment

The role of bioengineering

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Abbreviations: FDA, Food and Drug Administration; IFN-α2a, interferon-α2a; IFN-α2b, interferon-α2b; TSAs, tumor-specific antigens; TAAs, tumor-associated antigens; VLP, viral-like particle; HLA, human leukocyte antigen; mAbs, monoclonal antibodies; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; HER2/neu, human epidermal growth factor receptor 2; CTLA-4, cytotoxic T-lymphocyte antigen 4; CLL, chronic lymphocytic leukaemia; dAbs, domain antibodies; VEGFR, vascular endothelial growth factor receptor; P1GF, placenta growth factor; CTCL, cutaneous T-cell lymphoma

Cancer is one of the most important health problems because many cases are difficult to prevent. Cancer still has unknown mechanisms of pathogenesis, and its capacity to produce temporary or permanent damage, besides death, is very high. Although many anticancer therapies are available, finding a cure for cancer continues to be a difficult task. Thus, many efforts have been made to develop more effective treatments, such as immunotherapy based on a new class of tumor-specific products that are produced using recombinant DNA technology. These recombinant products are used with the main objectives of killing the tumor and stimulating immune cells to respond to the cancer cells. The principal recombinant products in anticancer therapy are immunostimulants, vaccines, antibodies, immunotoxins and fusion proteins. This review focuses on the general aspects of these genetically engineered products, their clinical performance, current advances and future prospects for this type of anticancer therapy.

Introduction

Cancer is one of the most common causes of death in humans. This condition is mainly characterized by the uncontrolled and invasive growth of cells, which may potentially spread to other parts of the body through the blood and lymphatics in a process called metastasis.

The most common cancer treatments are restricted to surgery, conventional chemotherapy and radiotherapy. Although these conventional anticancer therapies are effective in the management of many patients, these therapies are ineffective for approximately half of cancer sufferers.[1] Thus, new strategies are being developed and used to treat cancer by improving, supplementing or replacing conventional methods.

Recent advances have led to the development of an approach to cancer therapy known as immunotherapy. This therapy includes a variety of treatments based on recombinant products and/or engineered cells that have two main purposes: to stimulate the immune system and to reverse the tolerance that is provoked by cancer cells. Functioning as an immunostimulant, this therapy can improve effector cell maturation/activation and increase antigen priming and the delivery of immune cells to lymphoid and tumor tissues. Examples of this approach include cancer vaccines, engineered cytotoxic T cells and engineered immunocytokines. Tolerance-reversing approaches seek to suppress mechanisms related to tumor-associated immune tolerance, such as via immune suppressor cells, immune inhibitors and enzymes. Moreover, there are methods that can alter the tumor microenvironment using fusion proteins that release chemokines, costimulatory ligands and adjuvants (i.e., immunomodulatory molecules) and that can also function as an effective means of reversing tolerance.[2]

This article reviews recombinant products designed for anticancer therapy, mainly focusing on those products approved by the Food and Drug Administration (FDA), from the first technology until the most recent ones. Information is provided related to the products' structure, production and therapeutic indications; the human body's response to the products; and the challenges to achieving more pharmacological success. Additionally, future perspectives in this field are discussed, in which bioengineering is a fundamental tool for the development of immunotherapies.

Immunostimulants

Immunostimulants are cytokines that help the body to resist viral infections and cancers. The development of products that stimulate immune cells of either the adaptive or the innate immune

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response provides evidence for the capabilities of immunotherapy after a tumor has grown to the point of causing clinical disease.[3] Moreover, interferons, a class of cytokines with multifunctional properties, can induce pro-apoptotic gene expression, causing direct effects on cancer cells and inhibiting angiogenesis.[4] Of the three interferon types discovered, only type I interferons, and specifically interferon-α (IFN-α), have applications in anticancer therapy.

The first recombinant drugs developed were interferon-α2a (IFN-α2a) and interferon-α2b (IFN-α2b), with approval in 1986. These drugs' therapeutic indications were for the treatment of hairy cell leukemia, Kaposi sarcoma, chronic myeloid leukemia, malignant melanoma and follicular lymphoma (of the IFN-α drugs, the last two cancers are only treated with IFN-α2b) (**Table 1**). The compounds are produced from *Escherichia coli* strains. Although these medicines are associated with low response rates and toxicity at high doses, a restricted group of patients with a predisposition to autoimmunity has shown a good survival response.[5]

In addition to the direct effects of IFN-α, this drug has recently been used as an adjuvant to produce anticancer vaccines. IFN-α is capable of promoting the differentiation of human monocytes into dendritic cells (DCs) that present cancer cell antigens to T cells, which triggers an immune response.[6,7] Thus, scientists have taken advantage of that property to develop DC vaccines whose adjuvant is IFN-α.

The next recombinant drug approved by the FDA was interleukin-2, also known as aldesleukin (**Table 1**), which is also produced from *Escherichia coli*. Interleukin-2 plays a role in activating the production and stimulating the expansion of T cells[8] and has indications for the treatment of metastatic renal cell carcinoma and metastatic melanoma. Although this recombinant cytokine presents low complete response rates of approximately 15%,[5] the response is durable (approximately 10 y) in that specific group of patients.[8] Furthermore, there is a significant risk of systemic inflammation that requires interleukin-2 administration to be an inpatient procedure.

Vaccines

Active immunotherapy against cancer is represented by vaccines. Most vaccines aim to enhance or provoke an immune response against a tumor by means of tumor antigen-specific cytotoxic T lymphocytes (CTLs) because these cells are able to directly kill malignant cells.[9] Research on cancer vaccines has used several sources of tumor antigens, such as purified or synthesized tumor cell-surface molecules (proteins, peptides or lysates) and cells or lysates of allogeneic or autologous tumor cell lines.[10]

Tumor-specific antigens (TSAs) could be a perfect target for cancer vaccines because these antigens are essential for tumorigenesis and cancer progression. In contrast, tumor-associated antigens (TAAs) are not specific and can be found in tumors with the same histology as well as in tumors of different origins and even in certain normal cells.[11] Unlike TSAs, TAAs trigger only a weak immunological response due to self-antigen tolerance.[12]

Recombinant subunit vaccines are produced in genetically modified heterologous systems and have a number of advantages over traditional products, including increased specificity of action, reduced antigenic competition and greater safety.[13] Different types of cancer vaccines include recombinant live (viral and/or bacterial) vector vaccines, nucleic acid (DNA and/or RNA replicon) vaccines, protein and peptide vaccines, viral-like particle (VLP) vaccines, whole-cell vaccines (DC- or tumor cell-based), edible vaccines and combined approaches (e.g., prime-boost vaccination).[14,15]

Certain vaccines comprise autologous or allogeneic tumor cells that are removed by surgery and treated in the laboratory, generally using radiation (to avoid neoplasia formation). In certain cases, the cells are modified by adding chemicals or new genes so that the immune system recognizes these cells as foreign, after which the cells are injected into patients.[7] Among the advantages are the fact that such vaccines contain the necessary antigens to stimulate an antitumor response and do not require knowledge of which precise antigen to choose. However, tumor cell vaccines have the potential to cause autoimmunity and to increase the anergic status of T cells due to the absence of costimulatory molecules on the tumor cells.[16]

Cancer vaccines can also be based on single proteins or combinations of proteins, including heat shock proteins, peptides, anti-idiotype antibodies and fusion proteins. Among the advantages of these vaccines are that their production, storage and distribution are faster and that their cost-effectiveness is higher in comparison with tumor cell-based vaccines.[12] Additionally, TSAs are preferable because these antigens are able to produce a more individualized immune response to the tumor. However, these vaccines can initiate an autoimmune reaction, so certain types of HLA (human leukocyte antigen) restrict the vaccines' use, in addition to the weak immunogenicity of a single protein and the small capacity for evenly activating CD4 and CD8 receptors.[11]

Vector-based vaccines are based on the principle of mainly using viruses, bacteria or yeast to introduce recombinant genes, such as genes expressing TAAs, cytokines or costimulatory molecules, into APCs.[11] This method stimulates the APCs to produce an immune response against the tumor. For each type of vector, there are advantages and disadvantages, but generally, these vectors make it possible to insert an entire tumor antigen gene or its fragments or multiple genes and to infect professional APCs. In addition, the vectors have a lower cost of production than proteins or whole-tumor cell vaccines. Nevertheless, certain vectors can provoke an immunological reaction against themselves. The major vectors in use are vaccinia virus, adenovirus, *Saccharomyces cerevisiae*, *Salmonella* and *Listeria monocytogenes*.[17]

DNA vaccines are produced based on the capacity of vectors to transport DNA that encodes protein antigens and to insert this DNA into immune cells, which instructs the cells on how to initiate a desired response against a tumor.[7] This type of cancer vaccine has presented many advantages, such as the possibility of mobilizing both the cell-mediated and the humoral arms of the immune system in animals; easier and less expensive production than protein-based vaccines; and transgene expression that is thought to happen over a long period of time, which could obviate

repetitive booster vaccinations. Nevertheless, in early clinical trials, DNA vaccines have not adequately induced a robust immune response, demonstrating low immunogenicity.[18,19]

DCs have the strong ability to destroy cancer cells and to present antigens to CTLs. Because of that ability, these cells have been used to produce vaccines through the removal of a patient's DCs, whose expansion is promoted by immunostimulants in the laboratory, followed by contact with tumor antigens. By the end, this collection of cells and peptides is injected into the patient's bloodstream.[16] Although DCs are considered to be the most attractive means of immunization, this method has a high cost, and great efforts are involved in DC production.[17]

Cancer vaccines either prevent infection by cancer-causing viruses or the development of cancer in certain high-risk individuals (known as prophylactic cancer vaccines), or they treat existing cancer (known as therapeutic cancer vaccines).[20] A challenge in making a prophylactic vaccine is that there are many strains of virus.[3] However, certain prophylactic vaccines, such as the human papillomavirus (HPV) vaccine, have already shown good results. These vaccines are mainly designed to recognize carcinogenic etiologic agents, such as the hepatitis B virus, which can cause hepatocellular carcinoma. The HPV vaccine, consisting of a recombinant L1 protein that forms a VLP, is strain specific and intended to prevent approximately 70% of cases of cervical cancer by preventing infection with just two oncogenic strains, HPV 16 and 18.[21] Cervarix MEDI 51 and the quadrivalent HPV vaccine are examples of vaccines used to prevent HPV-associated cancer. Furthermore, the second is used to prevent genital warts caused by HPV 6 and 11 and is expressed in yeast, whereas Cervarix is expressed in baculovirus (**Table 1**).[22]

Therapeutic vaccines mainly aim to prime antigen-specific T cells and reprogram memory T cells, effectively transforming one type of immunity into another (e.g., regulatory to cytotoxic).[23] Nevertheless, therapeutic vaccine engineering may encounter several barriers, including the incomplete knowledge of tumor physiopathology and the variable immune response to antigens.[5] A therapeutic vaccine that is currently on the market is sipuleucel-T, designed to treat castrate-resistant (hormone-refractory) prostate cancer (**Table 1**). This vaccine is composed of many types of leukocytes, such as monocytes, T and B lymphocytes and macrophages; because of this complex composition, the vaccine's precise mechanism of action is unknown.[10] Sipuleucel-T received FDA approval after 225 patients experiencing advanced metastatic androgen-independent prostate cancer survived approximately four months longer than the control group in a clinical trial.[24] The success of sipuleucel-T, despite the side effect of flulike symptoms and the expensive cost, may only be the start of a wave of successes in the area of cancer vaccines.[25]

Antibodies

Currently, antibodies with a therapeutic aim are the most well-characterized proteins and one of the most successful and powerful tools that physicians employ to treat patients with cancer, inflammation or infectious diseases. Some of these antibodies boost the immune system once in the body, but other ones do not truly affect the immune system. Rather, these antibodies target specific parts of cancer cells, stopping the cells from growing or causing the cells to die.[7] Monoclonal antibodies (mAbs) are the class of antibodies that currently contribute most to recombinant products. The successful use of mAbs to treat a variety of cancers has demonstrated that even when one arm of the immune system is used in isolation, this method can be effective for the treatment of many types of cancers.[18,26]

Initially, hybridoma technology was used to produce mAbs. Only murine antibodies (human anti-mouse antibodies) were generated, which was problematic because the mAbs provoked clinic toxicity, triggered an immune response against themselves or were internalized with antigens from the cell surface and destroyed.[27] As a consequence of limited clinical utility, except for the FDA-approved I-131-anti-CD20 antibody tositumomab and the Y-90-anti-CD20 antibody ibritumomab tiuxetan, murine antibodies were not pursued further.[28] The first patient treated in the United States with an experimental mAb produced in mice, called AB 89 (designed to treat Hodgkin lymphoma), did not present a satisfactory clinical response. Because of this finding, the mAb was not approved by FDA, even though AB 89 has functioned as proof that it is possible to engineer antibody-based therapies.

The development of techniques to humanize or chimarize mAbs to decrease their murine components has been an important advance in the field of antibody therapeutics.[27] These new recombinant antibodies behave similarly to a naturally occurring immunoglobulin and mimic the normal antibody-based immune response, serving as effective agents in treating patients with cancer.[29] Current antibody targets are proteins that are unique tumor neoantigens, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), human epidermal growth factor receptor 2 (HER2/neu), specific antigens [e.g., cytotoxic T-lymphocyte antigen 4 (CTLA-4)] and molecular markers (e.g., CD20, CD52 and CD33).[5,18,26] The clinical successes of recombinant humanized therapeutic antibodies have included the improvement of overall survival and the time to disease progression in the treatment of human malignancies, such as breast, colon and hematological cancers.[30-33]

There are many hosts that can be used to produce mAbs, such as bacteria, yeast, plants and mammalian cells. Among these hosts, the last is the best host for antibody production, despite the elevated cost and the long periods necessary for cultivation. In contrast, there are processes that employ simpler, more cost-effective hosts and that are better suited to the production of antibody fragments, such as yeast and bacteria, but these hosts present glycosylation problems.[34] Moreover, bacteria possess simple expression systems that are frequently unable to make a recombinant human protein identical to the naturally occurring wildtype protein, and bacteria do not have refined mechanisms for performing posttranslational adjustments, which are present in more complex organisms.[35]

Plant use represents another recent strategy in the production of recombinant immunotherapeutics. Plants have high scalability, high cost-effectiveness, greater safety (plants do not carry mammalian pathogens) and the capacity to produce complex proteins

Table 1. Recombinant products approved by FDA for cancer therapy

Approval year	Drug	Drug class	Therapeutic indications	Organism class	Strains
1986	IFN-α2a	Immunostimulant-interferon	Hairy cell leukemia, Kaposi sarcoma	Human	*Escherichia coli*
	IFN-α2b	Immunostimulant-interferon	Hairy cell leukemia, Kaposi sarcoma, malignant melanoma, follicular lymphoma	Human	*Escherichia coli*
1992	Aldesleukin	Immunostimulant-interleukin	Metastatic renal cell carcinoma, metastatic melanoma	Human	*Escherichia coli*
1997	Rituximab	mAb	Non-Hodgkin lymphoma and CLL	Chimeric murine/human	Mammalian cell (Chinese hamster ovary)
1998	Trastuzumab	mAb	Metastatic breast cancer, gastric cancer	Humanized	Mammalian cell (Chinese hamster ovary)
1999	Denileukin diftitox	Immunotoxin	CTCL	Human	*Escherichia coli*
2000	Gemtuzumab ozogamicin	Antibody-conjugated	CD33-positive acute myeloid leukemia	Humanized	Mammalian cell
2001	Alemtuzumab	mAb	B-cell CLL	Humanized	Mammalian cell (Chinese hamster ovary)
2002	Yttrium-90 Ibritumomab Tiuxetan	Antibody-conjugated	Non-Hodgkin lymphoma	Murine	Mammalian cell (Chinese hamster ovary)
2003	Iodine-131 Tositumomab	mAb	CD20-positive, follicular, non-Hodgkin lymphoma	Murine	Mammalian cell
2004	Cetuximab	mAb	Metastatic colorectal cancer	Chimeric murine/human	Mammalian (murine myelo-ma) cell
	Bevacizumab	mAb	Metastatic colorectal cancer and HER2-negative metastatic breast cancer	Humanized	Mammalian cell (Chinese hamster ovary)
2006	Quadrivalent HPV	Vaccine	Cervical, vulvar, vaginal and anal cancer caused by HPV 16 and 18, genital warts caused by HPV 6 and 11	Viral	VLPs of the major capsid (L1) protein of HPV 6, 11,16 and 18
	Panitumumab	mAb	Metastatic colorectal carcinoma	Human	Mammalian cell (Chinese hamster ovary)
2009	Cervarix MEDI 501	Vaccine	Cervical cancer with HPV types 16 and 18	Viral	L1 protein of oncogenic HPV types 16 and 18, *Trichoplusia ni* insect cells
	Ofatumumab	mAb	CLL	Human	Recombinant murine cell line (NS0) using standard mammalian cell
2010	Sipuleucel-T	Vaccine	Castrate-resistant (hormone-refractory) prostate cancer	Human	Patient's peripheral blood mononuclear cells
2011	Ipilimumab	mAb	Unresectable or metastatic melanoma	Human	Mammalian cell (Chinese hamster ovary)
	Brentuximab vedotin	Antibody-conjugated	Hodgkin lymphoma, systemic anaplastic large-cell lymphoma	Chimeric murine/human	Mammalian cell (Chinese hamster ovary)
2012	Pertuzumab	mAb	HER2-positive metastatic breast cancer	Humanized	Mammalian cell (Chinese hamster ovary)
	Ziv-aflibercept	Fusion protein	Metastatic colorectal cancer	Human	Mammalian cell (Chinese hamster ovary)

in the right form (correct folding and posttranslational modification) and with the desired biological function.[36] However, studies on plant-based antibodies have indicated that there are still drawbacks regarding expression, proteolytic degradation and subcellular localization, which do not allow the antibody to be correctly secreted.[37]

The first mAb designed by a biomolecular engineering approach and approved by the FDA in 1997 was rituximab

(**Table 1**). This drug is a chimeric mAb targeting the CD20 antigen found in both normal B cells and most low-grade and certain higher-grade B cell lymphomas. Rituximab is indicated for the treatment of human lymphomas and chronic lymphocytic leukemia (CLL).[38,39] This drug is effective as a single agent in induction and maintenance therapy and also in combination with standard chemotherapies.[27]

One year later, trastuzumab was launched and is mainly indicated for the treatment of metastatic breast cancer (**Table 1**). Similar to which happens with the use of other recombinant medicines, patients in use of trastuzumab can develop resistance to that drug.[40] Therefore, a manner of increasing these drugs' effectiveness is to combine the drugs with other therapies, such as chemotherapy and radiotherapy. Pertuzumab (**Table 1**) is another recombinant agent that can be combined with drugs used to metastatic breast cancer. The efficacy and safety of the combined use of pertuzumab and trastuzumab in women with locally advanced, inflammatory or early HER2-positive breast cancer were evaluated in a randomized multicenter, open-label phase II trial, which demonstrated a favorable safety profile and the eradication of tumors in a proportion of these patients.[41]

An exception to that trend of drugs combined use is alemtuzumab, engineered for B-cell CLL (**Table 1**) treatment and used alone due to the major risk of infection if combined with chemotherapy.[27] In previously untreated patients with B-cell CLL, alemtuzumab alone produced an overall response rate of 83%, compared with 55% for the single agent chlorambucil (a chemotherapy drug), extending the time until alternative treatment from 15 to 23 mo.[42] Currently, ofatumumab (**Table 1**) is also indicated for the treatment of CLL; however, this drug is only used when alemtuzumab has failed.

In 2000, gemtuzumab ozogamicin (**Table 1**) was introduced onto the market. This drug consists of a humanized antibody conjugated to calicheamicin (a potent antibiotic isolated from *Micromonospora echinospora*) and raised against CD33, whose marker is positive in acute myeloid leukemia. A post-approval clinical trial with this drug was started but had to be stopped early because there was no improvement in the clinical benefit and greater toxicity was observed in the patients who received gemtuzumab ozogamicin than in the patients who had received only chemotherapy. Due to that finding, the drug was voluntarily removed from the market in June 2010.[43]

Since then, other conjugated antibodies have emerged. Yttrium-90 ibritumomab tiuxetan and iodine-131 tositumomab are radioimmunoconjugates directed against CD20 and designed for the treatment of non-Hodgkin lymphoma (**Table 1**).[27] The first antibody is a murine IgG1 conjugated to the radioisotope yttrium-90, whereas the second is a murine IgG2a radiolabelled with iodine-131. These murine antibodies were chosen because their half-life in humans is short, which reduces the systemic myeloablative side effects of the radioisotopes.[44]

Another conjugated antibody is brentuximab vedotin (**Table 1**), composed of anti-CD30 and an antimicrotubule agent (monomethylauristatin E) and intended for Hodgkin lymphoma and systemic anaplastic large-cell lymphoma treatment.[45] A single trial with this drug was performed with 102 patients with Hodgkin lymphoma and demonstrated that 74% of the patients had an either complete or partial response, and on average, the response to the therapy persisted for six to seven months.[43]

Bevacizumab is a humanized mAb indicated for the treatment of solid tumors, such as advanced colorectal cancer and lung, kidney and breast cancers (**Table 1**). This drug decreases tumors' blood supply by acting on VEGF. Another mAb, ipilimumab, whose target is CTLA-4, is indicated for the treatment of unresectable or metastatic melanoma (**Table 1**). In a pivotal phase III trial, ipilimumab use caused significant improvement in overall survival, and the drug presents new paradigms in terms of treatment-related toxicity. Ipilimumab's side effects are inflammatory and largely confined to the skin and gastrointestinal tract, but with an appropriate diagnosis, these effects can be manageable.[46]

Cetuximab, a chimeric antibody, and panitumumab (completely human) target EGFR (**Table 1**). The first antibody is used to treat head and neck cancers, and the second is used to treat refractory colorectal cancer.[47-52] Both antibodies face challenges in the clinic, such as the absence of reliable markers for the identification of patients who benefit from therapy with anti-EGFR mAbs, the short duration of the response in several patients and an elevated cost.[43]

The clinical success of therapeutic antibodies is demonstrated by 13 therapeutic mAbs that have received FDA approval for the treatment of a variety of solid tumors and hematological malignancies[46] and by a large number of additional therapeutic antibodies that are currently being tested in early- and late-stage clinical trials.

Additional strategies to generate entirely human antibodies have included phage display techniques and the use of transgenic mice to produce these antibodies. One of the problems that affects the efficacy of recombinant drugs is size. In general, the drugs are large molecules, rendering it difficult to penetrate through tumor tissues. Thus, innovative antibody engineering approaches to producing smaller antibody variants, fusion proteins and two-domain antibodies (dAbs) have also been utilized.[53-55] A dAb may be constructed from the variable domain of an antibody heavy chain or light chain.[56] dAbs are small, ranging from 12 to 15 kDa, monomeric, highly soluble, stable in circulation and easily engineered (making it possible to penetrate the blood-brain barrier) and have good expression in bacteria, yeast and mammalian cells.[57]

Another antibody class that has gained popularity is bispecific antibodies. These antibodies' principal characteristic is the capacity to link to two different epitopes or two antigens. Bispecific antibodies are made in several formats, such as bispecific single-chain variable fragments (ScFvs), tandem ScFv fragments (TaFvs) or bispecific and tandem diabodies, which target different antigens.[58] Among the antibodies' several applications, the following are of note: the recruitment of effector cells (e.g., CTLs, NK cells, macrophages and granulocytes) and the transit of systems (e.g., viral vectors) to target cells, where the antibodies can execute their action.[59]

Although recombinant antibodies are molecularly targeted therapeutic agents and represent a major new class of drugs for cancer treatment, there are a number of limitations to and issues

in the development of these antibodies, such as the high cost and the long time between engineering and approval. It is also important to note that certain patients treated with mAbs can develop resistance, so the improvement of the in vivo efficacy of therapeutic antibodies continues to be a challenge.

Immunotoxins

An immunotoxin is built from a combination of a highly selective cell ligand, known as a target moiety (such as an antibody or its fragments, a growth factor, a carbohydrate antigen or a tumor-related antigen),[60] and a toxin moiety that can be derived from bacteria, fungi, plants or human cells.[61] Nearly all immunotoxins act by inhibiting protein synthesis. Once the immunotoxins are internalized by the binding of the target moiety, only one toxin molecule is necessary to kill the cell. Because of this phenomenon, immunotoxins are very powerful agents.[62] Two main toxins used to produce these drugs are derived from *Pseudomonas* and *Diphtheria* bacilli.[58]

Immunotoxins can be used against solid and non-solid tumors but have a better response against the second because immunotoxins are large enough to pass through the tumor tissue. In addition, there are other difficulties in reaching major effectiveness, such as immunogenicity, toxicity (such as cardiac and gastrointestinal side effects) and the instability of the molecule. Certain alternatives created to overcome these difficulties include the use of an immunosuppressant, size reduction, the humanization of the components and the removal of certain toxin epitopes that are similar to B cell epitopes.[58] Currently, many clinical trials in phases I and II are being conducted to verify new immunotoxins' efficacy and security.

There is currently only one FDA-approved immunotoxin, called denileukin diftitox (**Table 1**), a fusion protein of interleukin-2 and *Diphtheria* toxin. This drug is used in therapy for recurrent cutaneous T-cell lymphoma (CTCL), but there are also many trials testing denileukin diftitox for the treatment of other types of cancer.[7,60] In a pivotal phase III study of patients with stage I-B to IV-A CTCL, this drug showed 30% clinical responses, including 20% partial responses and 10% complete responses lasting a median of six months.[42] Denileukin diftitox performance is limited because of its poor affinity for the IL-2 receptor, related to absence of CD122,[58] and denileukin can induce the development of a human antitoxin antibody response by the second treatment.[63,64]

Fusion Proteins

Most fusion proteins are engineered from a receptor extracellular domain and the Fc portion of a human immunoglobulin (generally IgG). These proteins can inhibit one or more receptors, act in tumor-associated angiogenesis, interfere in EGFR signaling and stimulate the recruitment of immune effector cells.[65] Moreover, fusion proteins easily traverse tumor tissues because the proteins are small.

Aflibercept (**Table 1**) is a recombinant protein resulting from a fusion of the immunoglobulin portions of vascular endothelial growth factor receptor (VEGFR)-1 and 2. This drug blocks angiogenesis by binding to human VEGF-A, VEGF-B and placenta growth factor (P1GF).[65,66] Aflibercept was approved by the FDA in 2012 under the name ziv-aflibercept due to its use in combination with a chemotherapy scheme comprising 5-fluorouracil leucovorin and irinotecan for the treatment of metastatic colorectal cancer. A phase III trial, named VELOUR, with this drug association presented a 24% decrease in the risk of metastatic colorectal cancer progression and an improvement in the response rate from 11% to 19.8% and in survival at 2 years from 19% to 28%.[67]

Future Perspectives

From the 1980s until now, advances in anticancer therapy have been remarkable and have represented new hopes for patients with malignancies and with a poor life expectancy. Immunostimulants, such as IFN-α, were the first recombinant products that nonspecifically boosted the immune system to attack cancer cells. In addition to individual use, IL-2, for instance, is being employed as an adjuvant in cancer vaccines to increase the immune response. Currently, many formats of antibodies and their fragments are dominating the market due to high specificity and good clinical response, whether used singly or in combination with drugs, toxins or radionuclides. Moreover, cancer vaccines are promising in spite of many studies that did not show high effectiveness in humans. Therapeutic vaccines are the most challenging, but experts are developing techniques to obtain better results, such as by treating the patient's own cells in vitro and reinserting the cells into the bloodstream. Thus, cancer immunotherapy is progressively more individualized and is approaching a future in which there will not be just one solution to cancer but rather many solutions to cure or prevent malignancies.

These advances would not be possible without biotechnology support and especially bioengineering. Since the initial hybridoma technology was developed until more recent techniques, many recombinant drugs have been designed with more specificity, more effectiveness and fewer side effects. Furthermore, several organisms have been used to reach those objectives, such as bacteria, viruses, yeast, insects and plants. Although there are only 21 recombinant drugs approved by the FDA for anticancer therapy, many more are in clinical trials and should be on the market in the next few years, inaugurating a new era in cancer treatment.

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