Review

Recent advances in the field of anti-cancer immunotherapy

CrossMark

Henrique Neves, Hang Fai Kwok *

Faculty of Health Sciences, University of Macau, Avenida de Universidade, Taipa, Macau

ARTICLE INFO

ABSTRACT

Background: The main goal of anti-cancer therapy is to specifically inhibit the malignant activity of cancer cells, while leaving healthy cells unaffected. As such, for every proposed therapy, it is important to keep in mind the therapeutic index — the ratio of the toxic dose over the therapeutic dose. The use of immunotherapy has allowed a means to both specifically block protein–protein interaction and deliver cytotoxic events to a tumor-specific antigen.
Review scope: It is the objective of this review to give an overview on current immunotherapy treatment for cancers using monoclonal antibodies. We demonstrate three exciting targets for immunotherapy, TNF-α Converting Enzyme (TACE), Cathepsin S and Urokinase Plasmogen Activator and go over the advances made with one of the most used monoclonal antibodies in cancer therapy, Rituximab; as well as Herceptin, which is used for breast cancer therapy. Furthermore, we touch on other venues of immunotherapy, such as adaptive cell transfer, the use of nucleic acids and the use of dendritic cells. Finally, we summarize some ongoing studies that spell tentative advancements for anti-cancer immunotherapy.
General significance: Immunotherapy is at the forefront of anti-cancer therapies, allying both a high degree of specificity to general high effectiveness and fewer side-effects.
© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Contents

1. Introduction

The 21st century has ushered in an era of great scientific progress and discoveries, resulting in a surge of interest by the general public in

* Corresponding author. Tel.: +853 8822 4991.
E-mail address: hfkwok@umac.mo (H.F. Kwok).

all manner of research. Modern science looks to improve lives, focusing not only in the eradication of disease, but also in extending the average lifespan of humans. Unfortunately, as people live longer, new problems arise. As age increases, an individual is more likely to develop complications, namely degenerative diseases, such as cancer.

In cancer biology, tumors are described as complex tissues comprised of heterogeneous neoplastic cells interwoven with tumor-associated stroma. The characterization of proteins associated with tumors presents opportunities for targeted therapeutic intervention. This approach is called "targeted therapy". However, the heterogeneity of tumors dictates that, in order to achieve successful clinical treatment, it is necessary to employ a combination of targeted therapies. The most specific targeted therapies currently in use are monoclonal antibodies.

In the last decade, the use of antibody therapy in the field of oncology has shown very promising results [1]. Due to their high specificity, antibodies represent a promising method for interfering with a single target molecule, with high selectivity. Back in 1980, the first patient with relapsed lymphoma was treated using a therapeutic antibody approach. While the antibody was shown to be clinically ineffective, the therapy was deemed innocuous and was well-tolerated [2,3]. These safety and tolerated rationales built up the groundwork that led to the use of therapeutic antibodies in the treatment of cancer.

During the past few years, attention has turned to using antibodies to target different tumor-associated antigens. These include surface glycoproteins associated with clusters of differentiation, CTLA-A, or pathways regulated by growth factors [4]. Furthermore, while the use of monoclonal antibodies monotherapy has had a tremendous impact on cancer treatment, namely in non-Hodgkin's lymphoma, their efficiency has been further improved through the combination of chemotherapy along with monoclonal antibodies [5].

However, many of the studies presented ambiguous or insufficient criteria for clinical objective response. Results from such studies may improperly imply effectiveness when compared to historical controls. This emphasizes the need for thoughtful changes in the application of cancer treatment approaches, such as a combination of multi-targeting antibody-based therapy [6–8].

2. Monoclonal antibody immunotherapy

One of the most promising and exciting fields in modern anti-cancer therapy involves the use of monoclonal antibodies which, once administered to the patient, will selectively and efficiently, target a particular protein involved, in some way, with the proliferation of tumor cells. A large number of monoclonal antibody therapies have already been approved and are currently in use, as described in Table 1.

In the cases described below – TACE/ADAM17, Cathepsin S and Urokinase Plasminogen Activator – the proteins show an abnormally high expression in cancer cells. This makes them the perfect targets for inhibition through the use of monoclonal antibodies.

Furthermore, we also take a look at Rituximab, one of the principal antibodies used in anti-cancer therapy, as well as Herceptin, the only antibody therapy approved by the FDA that targets the human epidermal growth receptor 2 protein.

2.1. TNF-α Converting Enzyme (TACE)

Many growth factors and cytokines require proteolytic release from the cell surface for their activation [11]. TNF-α converting enzyme (TACE), also known as A Disintegrin and Metalloprotease 17 (ADAM17); is a transmembrane metalloprotease responsible for solubilizing many pathologically significant membrane substrates and is an appealing therapeutic target for the treatment of several diseases [11]. In terms of structure, mature ADAM-family ectodomains contain a globular metalloprotease catalytic domain, a disulfide-dependent disintegrin-cysteine rich (Dis-Cys) domain and, in some cases, an epidermal growth factor (EGF)-like domain [11].

Initially, TACE was described as an enzyme, whose function was attributed to solubilizing membrane-associated pro-TNF-α [12] — a process named "ectodomain shedding". Since then, TACE has been described as capable of cleaving epidermal growth factor receptor (EGFR) ligands [13,14], extracellular Notch1 [15], adhesion molecules [16] and cell-surface receptors [17]. Ever since proteolytic cleavage has been proven to be indispensable for the activation of many of these substrates, TACE has been studied as a target in the treatment of cancer [18] and rheumatoid arthritis [19]. Furthermore, dysregulation of ectodomain shedding has been linked to autoimmune and cardiovascular diseases, neurodegeneration, infection and inflammation [20].

Several studies have demonstrated that TACE is over-expressed in various tumor cells, such as those from ovarian cancer, breast cancer, pancreatic ductal adenocarcinoma, colorectal carcinoma, gastric cancer stem cells, gastrointestinal stromal tumors (GIST), non-small cell lung carcinoma and head and neck cancer [21]. This protein has also been associated in governing endothelial cell migration and pathological angiogenesis, which are equally relevant to tumor growth [21]. Chemotherapy may activate TACE, leading to growth factor shedding, which contributes to resistance in colorectal cancer models, as well as contributing to resistance to trastuzumab in breast cancer [21].

There is a very high homology (96%) between the human and mouse TACE ectodomains, which makes the antibody selection and production process even more important. Adding to that, there is the need to adhere to the therapeutic requirements for human antibodies and the desire to avoid metzicin active site immunoreactivity. For these reasons, antibody phage-display presents an attractive technology for producing a specific TACE inhibitor [22].

Antibody phage-display is a powerful *in vitro* selection technology capable of producing fully human antibodies against human antigens. A flowchart of the main steps in phage-display technology is present in Fig. 1.

This technique can be used to direct antibodies towards desired epitopes, due to the biochemical control available during selection conditions. Solution-phase phage display typically produces antibodies with non-linear (conformational) epitopes. Thus, intricate macromolecular cross-domain binding might be hypothetically achieved through this technology, an ideal scenario for an ADAM inhibitor [11]. In fact, antibodies have been produced through phage display, due to recent technical advances, capable of recognizing multiple distinct antigens [23] and different conformations of the same antigen [24,25].

Table 1
Monoclonal antibodies currently in use in anti-cancer immunotherapy, targets and respective cancer types, as well as date of approval both by the Food and Drink Administration and by the European Medicines Agency.
Adapted from Chames, P., et al. Br. J. Pharmacol, 2009 [9] and Oldham, R. K. and Dillman, R. O. Journal of Clinical Oncology, 2008 [10].

Monoclonal antibodies used in cancer immunotherapy					
Generic name	Commercial name	Target	Cancer type	FDA approval	EMEA approval
Rituximab	Rituxan	CD20	Non-Hodgkin's lymphoma	26/11/1997	2/6/1998
Trastuzumab	Herceptin	Erb B2 (HER-2)	Breast	25/9/1998	28/8/2000
Alemtuzumab	Campath	CD52	Chronic lymphocytic leukemia	7/5/2001	6/7/2001
Cetuximab	Erbitux	EGFR	Colorectal	12/2/2004	29/6/2004
Panitumumab	Vectibis	EGFR	Colorectal	27/9/2006	19/12/2007
Bevacizumab	Avastin	VEGF	Colorectal	26/2/2004	12/1/2005
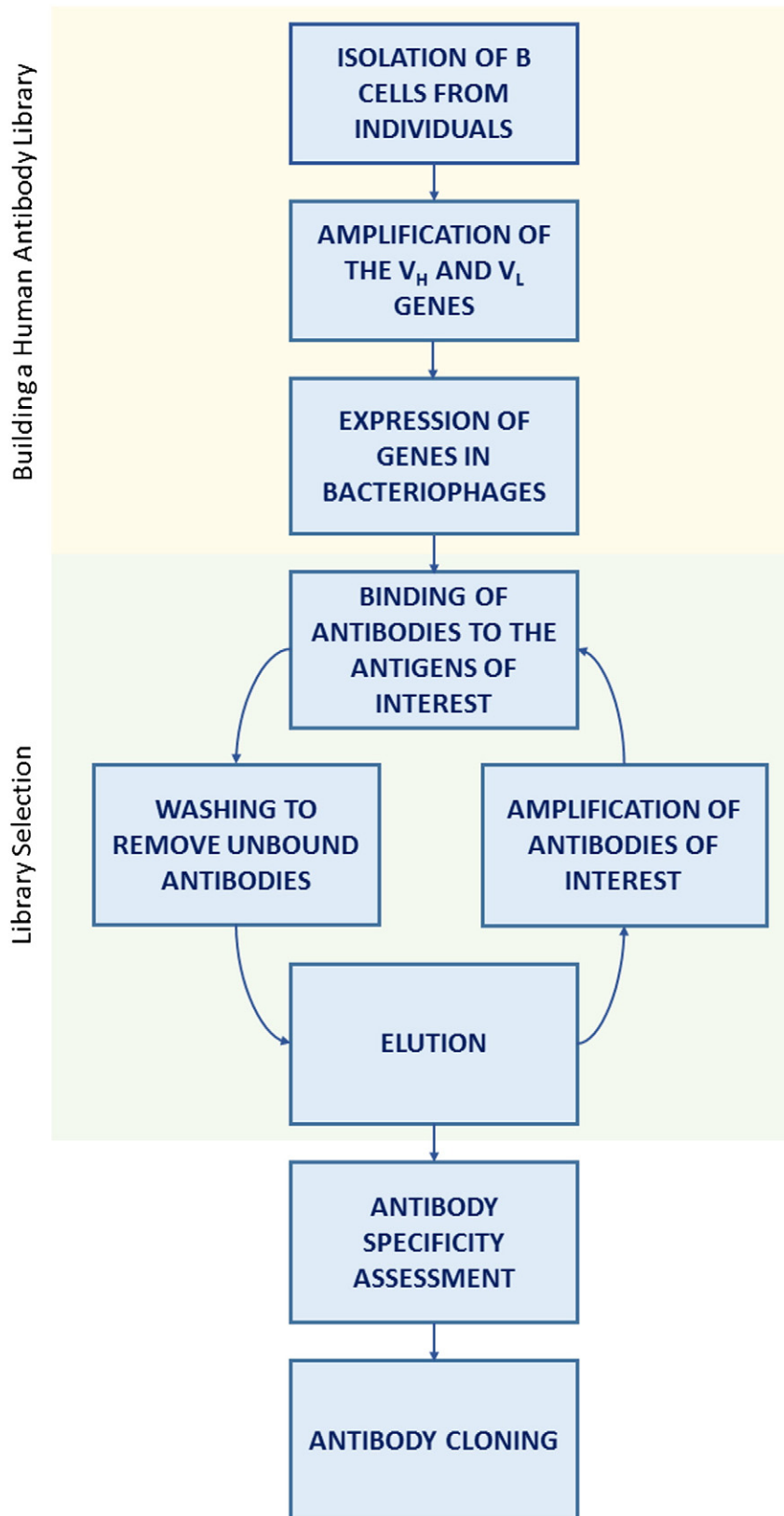
Fig. 1. Flowchart for the protocol for Phage Display Technology. V_L and V_H refer to variable light and variable heavy chains in antibodies. Various genes responsible for encoding the variable regions of antibodies are amplified from human B-cells and used to build an antibody library. The library is cloned for display on the surface of the phage. In a procedure similar to the two-hybrid system, the antibody fragment is expressed in fusion with the virus coat protein. The phage display library goes through a process of selection, whereupon those that do not bind to the selected epitopes are washed away. The ones that do are eluted and amplified by infection of *Escherichia coli*. After an adequate number of selection series, the specificity of the desired antibody can be assessed through Enzyme-Linked Immunosorbent Assay (ELISA) or Fluorescent-Activated Cell Sorting (FACS). Once the achieved specificity is satisfactory, the genes corresponding to the antibody's variable regions can be cloned into whole human IgG expression vectors and transfected into cells, such as HEK293, which will produce fully human mAbs (hmAbs). The antibodies will be expressed into the cell medium. At that point, the supernatant can be collected for antibody purification.

D1(A12) is a monoclonal antibody developed through antibody phage-display, which targets the TACE ectodomain. Studies have allowed a comprehensive understanding of the biochemical properties of D1(A12), through the use of assays on human cancer cells. Furthermore, it has been confirmed through xenograft analyses, in *in vitro* as well as *in vivo*, that D1(A12) serves as a potent inhibitor of human ADAM17's activity [11,21]. However, later studies have demonstrated that this antibody was unable to lower the concentration of human TNF-α circulating in the bloodstream. These results suggest that, following the inhibition of ADAM17 in an *in vivo* environment, other factors may replace the concentration of TNF-α [21]. One possible culprit is ADAM10, as this enzyme has shown sheddase activity towards TNF-α in murine fibroblasts that were deficient in ADAM17. In certain types of lymphoma, ADAM10 is also responsible for the solubilization of TNF-α [21]. Recently, it was determined that the D1(A12) antibody can successfully inhibit the proliferation and motility of cancer cells in head and neck squamous cell carcinoma (HNSCC), by reducing the overall amount of circulating EGFR ligands [26]. These results further prove, not only the promising future applications of this particular antibody in cancer therapy, but also the importance of cancer immunotherapy, moving forward.

Studies continued, in an effort to identify an antibody possessing cross-reactivity between human and mouse antigens. This is important, particularly in pre-clinical trial conditions, to ensure the safety of the proposed therapy. Thus, a method was proposed that alternates selection rounds between human and mouse antigens [22]. The discovery of such an antibody would allow research to proceed into a purely *in vivo* environment. With these conditions in mind, work continued, resulting in the identification of A9, an antibody clone that demonstrated mostly non-competitive inhibition [22].

Subsequent experiments revealed that A9 was an allosteric inhibitor, which could bind to a secondary site outside the catalytic cleft of TACE, thus disturbing its ability to bind to the active site [22]. In fact, experiments developed in the presence of CT1746 – a hydroxamate inhibitor of metalloproteinases that interacts with TACE's active site Zn [26] – demonstrated that the binding of ligands to the active site of TACE affected the A9 binding site on the protein. In other words, the affinity of A9 to TACE was reduced in the presence of CT1746 [22]. This data suggests that the inhibition of TACE by A9 is not purely non-competitive, but rather a mixed form of inhibition.

It is important to consider that there are approximately 70 known metzincin metalloproteases that possess Zn in their active site [27]. Therein lies the problem of small molecule inhibitors of TACE: the lack of selectivity in these inhibitors would lead to off-target toxicity [28]. Hence, the significance of A9: a non-Zn-binding inhibitor, specific for the TACE protein.

Due to the importance of this protein in a cancer environment and the promising results described above, this area and, in particular, TACE inhibition; has proven itself to be rife with possibilities on the path of cancer research and eventual eradication.

2.2. Cathepsin S

Another promising target being investigated is Cathepsin S, a proteolytic enzyme. This protein functions predominantly as an endopeptidase within the endolysosomal vesicles of healthy cells, and is involved in many physiological processes, such as differentiation, protein turnover, degradation and apoptosis. In many cancer cell lines, Cathepsin S has been demonstrated to be highly expressed or upregulated, contributing to the development and progression of the cancer phenotype [6].

In colorectal cancer patients, Cathepsin S associates with the cell membrane, providing an opportunity for antibody-dependent cellular cytotoxicity. In fact, the targeting of Cathepsin S, in this case, through the use of a humanized antibody with an immune effector function, has resulted in natural killer cell targeted tumor killing, with a 22% cytotoxic effect [29,30]. Furthermore, by selectively targeting Cathepsin S,

the antibody treatment inhibits the breakdown of the extracellular membrane around the extracellular periphery of tumor cells, resulting in the attenuation of tumor cell invasion through the extracellular membrane. This leads to an inhibition of tumor cell invasion, growth and neovascularization [29].

A recent antibody, Fsn0503h, has been developed which can inhibit Cathepsin S. The *in vivo* results are promising, and include the suppression of angiogenesis and metastasis, effectively halting cancer progression. While there is still much to be uncovered regarding this lysosomal cysteine protease, Cathepsin S is thought to contribute to resistance against more common types of cancer therapy, such as radio and chemotherapies, making it an important research subject in the field of immunotherapy [34].

2.3. Urokinase Plasminogen Activator

Mammary carcinoma and lung cancer are the most common type of malignant tumors in adult women and an undeniable concern for general public health. Unfortunately, some of the issues with finding a solution to human breast cancer include its high genetic heterogeneity, different molecular profiles and varied clinical behavior. One of the targets being focused on, in an attempt to eradicate this type of tumor, is the urokinase plasminogen activator protein [31].

The urokinase plasminogen activator (uPA) system is composed of uPA, a specific cell receptor for uPA (uPAR), and serpin inhibitors of uPA, such as plasminogen activator inhibitor-1. Among the roles of this system, are the release and processing of latent growth factors located on the extracellular membrane, such as FGF-2, VEGF, HGF and TGF-β. There is a great number of papers describing the crucial role of uPAR in the evolution of some solid cancers, including breast, colon, prostate, pancreatic, ovarian, lung and brain, as well as several hematologic malignancies such as acute leukemia and myeloma [31,32]. This has led to the recent identification and development of a monoclonal antibody that specifically targets uPAR. This therapy has proven effective in a number of different animal tumor models, without blocking the interaction between uPA and uPAR [32].

Recently, a novel therapeutic uPAR antibody was developed, ATN-658, which has been capable of exhibiting reliable anti-tumor effects across a variety of tumor models. ATN-658 has been proven to inhibit invasion, metastasis and tumor proliferation as well as induce apoptosis. In uPAR's DIII domain, there is a small 6-mer disulfide loop near the glycolipid anchor, which serves as the antibody's epitope. The antibody closely mimics the interaction of CD11b. CD11b-positive cells act as suppressors to diminish cytotoxic T-cell response, allowing tumors to progress, as well as secrete factors that drive that development. ATN-658 blocks the CD11b–uPAR interaction, which leads to the hypothesis that uPAR may actually function to promote metastasis [32].

Beyond the use of antibodies, a study has been described that uses aptamers, specifically RNA aptamers, which selectively bind to human uPA, by targeting the active site of the enzyme. This inhibition effectively halts the activation cascade of pro-uPA, while not interfering with any uPA that are already active, or even other serine proteases. The effects of using the above aptamer, named upanap-126, include a reduction in tumor dissemination and cell invasion [37].

As a result, the further study of the uPA system may be an important future endeavor in the fight against cancer.

2.4. Rituximab

One of the existing anti-cancer therapies that has found some success, is the monoclonal antibody Rituximab.

Rituximab, also known as IDEC-C2B8 [33], is used for treatment directed against the B-cell-specific antigen CD20 expressed on non-Hodgkin's lymphomas. It revolutionized the clinical treatment of B-cell non-Hodgkin's lymphoma, being the first monoclonal antibody drug to be approved, in 1997 [34]. It is a chimeric human–mouse monoclonal

antibody that specifically binds to the CD20 antigen on the surface of normal and tumor B-cells [33,34]. As CD20 is absent from hematopoietic stem cells, normal B-cells are able to regenerate after the Rituximab treatment and return to pretreatment levels within several months or years [35].

However, treatment with Rituximab has been linked to moderate to severe first-dose side-effects, notably in patients with high numbers of circulating tumor cells [36]. These side-effects include fever, rigors, bronchospasm and hypoxemia, concomitant with rapid reduction and laboratory evidence of tumor destruction [37]. In some cases, tumor lysis syndrome has been detected in the 24 h period following the first infusion with the antibody. This condition is characterized by a rapid reduction of the tumor followed by acute renal failure, hyperkalemia, hyperuricemia, hyperphosphatemia, hypocalcemia and, on occasion, death. Other risks include a high number of circulating malignant cells or high tumor burden. Should tumor lysis occur, electrolyte imbalances should be corrected while monitoring renal functions and fluid balance [38].

When considering patients with autoimmune diseases, it is important to keep in mind the unknown, but possible development of malignancies with the administration of Rituximab. This is especially compounded in elderly patients, as the recovery to normal levels of B-cells can be delayed. Prolonged immunosuppression has been associated with increased incidence of cancer [39]. Even so, the use of Rituximab in autoimmune diseases is rapidly increasing [40]. While the efficacy and safety vary among different autoimmune diseases, the use of this treatment is ultimately beneficial, as is suggested by most studies [39].

In cases of idiopathic neuromyelitis optica, a demyelinating disease of the central nervous system characterized by the co-occurrence of transverse myelitis and optic neuritis, treatment with Rituximab was well tolerated and patients experienced less exacerbation than expected, based on their historical data for attack rates [41]. In the case of 35-year old woman, who had developed Burkitt's lymphoma during early pregnancy, treatment with Rituximab and CHOP therapy was safely administered, without causing any malformation, developmental retardation or immune dysfunction in the child, while producing complete remission in the mother [42].

It has been suggested that complement and complement inhibitors are likely to play a role in the heterogeneity of the response to Rituximab *in vivo* [43]. Administration of Rituximab results in a prompt activation of the complement system, resulting in cytokine release. This, in turn, activates macrophages and mast cells, which are capable of releasing cytokines themselves as well as complement activation products, which can function as anaphylatoxins, and might be thus responsible for some of the side-effects [36]. It is likely that the mechanism of action of Rituximab *in vivo* is to some extent affected by the complement. This means that studies to improve the safety as well as the efficacy of these treatments should keep in mind the role of the complement in the treatment [36,44].

2.5. Herceptin

However, Rituximab is not the only monoclonal antibody therapy in use today.

In 20% to 25% of invasive breast cancers, the human epidermal growth receptor 2 protein (HER-2) has been found to be overexpressed [45,46]. HER-2 is a tyrosine kinase that is related to the epidermal growth factor receptor EGFR [45]. Its structure is composed of an extracellular domain, a transmembrane domain and an intracellular domain with tyrosine kinase activity [46]. This protein has the ability to transform normal fibroblasts and, when overexpressed, produce breast cancer in transgenic mice. This enzyme has become an important therapeutic target in breast cancer, as higher levels are closely linked with higher pathogenesis and worse prognosis of breast cancer. Due to the fact that HER-2 is overexpressed mainly in tumor cells, the risk

of toxicity of HER-2 targeting drugs is decreased, as it is present in much higher proportion in said tumor cells, compared to healthy cells [45].

Herceptin, also known as trastuzumab, is a recombinant humanized monoclonal antibody, directed against the extracellular domain of the HER-2 protein. Currently, Herceptin is the only therapy approved by the United States Food and Drug Administration that targets HER-2. Before treatment with this mAb therapy, the American Society of Clinical Oncology recommends the evaluation of HER-2 status in all primary breast tumor, both at the time of diagnosis and upon recurrence, as this affords both prognosis information, as well as being determinant of the response to Herceptin [45].

Although the exact mechanisms by which Herceptin is capable of inhibiting HER-2 are not yet completely understood, some of its effects have been observed, both *in vitro* and *in vivo*, such as diminished receptor signaling, induction of apoptosis, inhibition of angiogenesis and inhibition of DNA repair [45].

Some of the mechanisms that may be used by therapeutic antibodies to combat cancer cells are exemplified in Fig. 2.

Initial phase I clinical trials with this antibody proved it to be safe and with reliable pharmacokinetics, giving response rates of up to 34%. A later study observed that combining Herceptin with doxorubicin plus cyclophosphamide produced longer time to progression, higher response rates and improved survival rather, as opposed to simple chemotherapy. However, this also caused severe cardiac dysfunction [48]. At the same time, research groups are attempting to identify new means of increasing Herceptin efficiency while decreasing cardiotoxicity. The solution might include a multidisciplinary care approach, with both cardiology and oncology specialists providing a risk–benefit assessment and proper patient education on how to manage the disease, the cure and on adopting a healthy lifestyle [49].

One of the main issues with this mAb therapy lies in the fact that objective response rates, when in a monotherapy regimen, are low, ranging from 12% to 34% for a duration of 9 months. Because of this, Herceptin is usually administered in combination with chemotherapies, such as paclitaxel or docetaxel, which increase response rates, time to disease progression and overall survival [50]. Unfortunately, patients who demonstrate an initial response to Herceptin-based regimens, generally acquire resistance within one year [45].

Some mechanisms have been proposed that explain how tumors avoid the cytotoxicity caused by this therapy. One such possibility are mutations in the *her2* gene (also called *erbB-2*), – which encodes for the HER-2 protein – resulting in an inability for the antibody to recognize its epitope and, therefore, to bind to HER-2 [45,51]. Another mechanism revolves around the fact that EGFR type I growth factor receptor tyrosine kinase family consists of EGFR, HER-2, HER-3 and HER-4. Although it is possible that Herceptin is, indeed, inhibiting cell signaling through HER-2 binding, it will not reduce signaling through the other HER receptors. Fortunately, new antibody therapies are being developed to counter this mechanism [45].

Moving forward, more research is being done with Herceptin in order to maximize its potential as an immunotherapy. This includes combining Herceptin with novel agents, such as the anti-EGFR tyrosine kinase inhibitor gentifibin, which has produced complete remission of BT474 breast tumor xenograft; as well as developing novel strategies for targeting HER-2, like the recombinant humanized HER-2 mAb pertuzumab, capable of blocking the dimerization of HER-2 with other HER receptors, thus avoiding one of the mechanisms described above through which patients develop resistance to Herceptin therapy [45].

Upon observing how much effort is being placed into ensuring the effectiveness of anti-HER based treatment, it is clear that this mAb therapy may have lost the battle, but is still far from losing the war. This is especially true if new active targeted agents are developed, namely against other members of the HER receptor family [51].
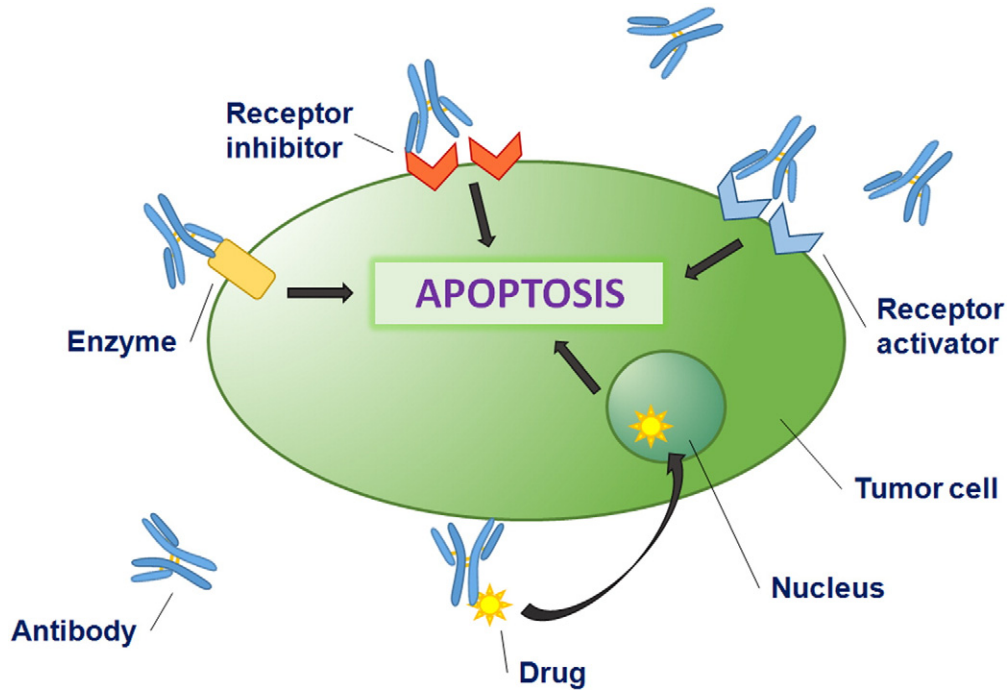
Fig. 2. Direct tumor cell killing by antibodies. This event may occur through receptor antagonist activity, which occurs when an antibody blocks dimerization, downstream signaling and kinase activation, resulting in inhibition of proliferation and, finally, apoptosis (such is the case with TACE/ADAM17 binding and the therapeutic antibody Herceptin). It can also be triggered by antibodies binding to a tumor cell surface receptor, leading to its activation and, consequently, apoptosis; which is represented by the mitochondrion. Finally, an antibody may bind to an enzyme, leading to signaling abrogation, neutralization and cell death. Conjugated antibody therapies are based around delivering a payload – for example, a drug, toxin, small interfering RNA or radioisotope – to a tumor cell.
Adapted from Scott, A. M. Nature Reviews Cancer, 2012 [47].

2.6. Antibodies reviews

However, Rituximab is not the only antibody currently being used or studied for therapy. Other targets and antibodies are being considered for therapeutic purposes.

One such case of a promising antibody is BMS-936558, also known as MDX-1106 and ONO-4538. This is a monoclonal antibody that targets a key immune-checkpoint receptor expressed by activated T-cells, programmed death 1 (PD-1). PD-1 functions primarily in peripheral tissues, where T-cells may encounter the immunosuppressive PD-1 ligands PD-L1 (B7-H1) and PD-L2 (B7-DC), which are expressed by tumor cells, stromal cells or both. Inhibition of the interaction between PD-1 and PD-L1 can enhance T-cell responses *in vitro* and mediate preclinical antitumor activity [52]. While the use of BMS-936558 was safe and well-tolerated, results on its effectiveness are still inconclusive [53,54].

Other therapeutic targets include Glypican-3, claudins and the B-cell maturation antigen.

Glypican-3 (GPC) is a member of the glypican family of heparan sulfate proteoglycans. It has presented itself as an attractive target for immunotherapy due to its overexpression in 72%–81% of hepatocellular carcinoma (HCC), the leading cause of cancer-related deaths worldwide. In these cases, immunotherapy has not had dramatic effects in patients with advanced HCC, suggesting that further analysis and knowledge of GPC3 biology are needed to allow the development of more effective GPC3-targeted cancer therapies. Research has entered clinical trials [55].

Claudins are tight junction proteins that are abnormally regulated in several human cancers. In particular, claudin-3 and claudin-4 are frequently overexpressed in several neoplasias, including ovarian, breast, pancreatic, and prostate cancers. Even though tight junction proteins have been studied for their role in tumorigenesis for many years, serial analysis of gene expression (SAGE) and array analyses of breast and ovarian cancers have identified claudins as proteins frequently altered

in cancer. These findings suggest a possible avenue for the detection, diagnosis and treatment of drug-resistant cancers [56].

Multiple myeloma is a neoplasm of plasma cells and it affects 1 to 5 per 100,000 individuals worldwide, with a higher incidence in the West [57]. Although there is no monoclonal-based targeted therapy approved to treat patients with multiple myeloma. However, a monoclonal antibody therapy is being developed that selectively targets the B-cell maturation antigen (BMCA), a member of the tumor necrosis factor receptor superfamily. Conjugation of this anti-BMCA mAb J6M0 to the potent microtubule disrupting monomethyl auristan E (MMAE) or F (MMAF) with protease cleavable valine-citrulline (vc) or uncleavable maleimidocaproyl (mc) linkers has yielded promising results. Particularly, J6M0-mcMMAF (GSK2857916) has shown rapid and sustained elimination of MM tumors in 3 different mice models, as well as inducing antibody dependent cell mediated cytotoxicity (ADCC) and antibody dependent cell phagocytosis (ADCP) [58].

3. Other forms of immunotherapy

Although monoclonal antibodies are at the forefront of immunotherapy, particularly due to their effectiveness and specificity, along with their safety of use; they are not the only forms of immunotherapy being investigated and applied. Below, we offer brief compendiums on each of the most prevalent immunotherapies available, which include adoptive cell transfer, and the use of nucleic acids and dendritic cells.

3.1. Adoptive cell transfer

Adoptive cell transfer refers to the stimulation of T-cells *ex vivo* by activating and expanding analogous tumor-reactive T-cell populations to large numbers of cells that can then be transferred back to the patient, or to a new recipient host, with the goal of transferring the immunologic functionality and characteristics into the new host [59].

Tumor-specific CD8$^+$ cytotoxic T lymphocytes can be generated through the use of peripheral blood mononuclear cells with tumor-associated antigen. These antigen-presenting cells, such as dendritic cells express certain cytokines, including interleukin-2, IL-7, IL-12, IL-15 and IL-21 [60].

Combining immunotherapy with either cytotoxic chemotherapy or targeted therapy can promote the therapeutic potential for the treatment of cancers in comparison with the use of either treatment alone [60]. Dying tumor cells release abundant antigen, which may induce cytotoxic chemotherapeutical agents to an increased effector cell capacity, to recognize and kill tumor cells [61]. The processing of the antigens that result from this event can lead to the priming of adoptively transferred cells, as well as the activation of endogenous tumor-specific T cells [60]. Combined with chemotherapy, this treatment can lead to the enhancement of anti-tumor immunity through increased tumor-specific immune responses *via* the cross-priming of apoptotic tumor cell death and shows to be beneficial for survival in a phase II trial in patients with newly diagnosed glioblastomas [62,63].

3.2. Nucleic acids

Recently, attention has turned to using nucleic acids directly. The therapies developed center around protocols that include utilizing small double-stranded RNA as an anti-tumor and anti-metastatic solution. Small interfering RNAs have been documented as inducing INF-α, but not proinflammatory cytokines, such as IL-6. Not only that, but sdsRNA has also induced the secretion of TNF-α, in cases of hepatocellular carcinoma. As such, it is considered a potential adjuvant for the therapy of immunosuppressive oncological diseases [64].

3.3. Dendritic cells

Dendritic cells are called the sentinels of the immune system. They are crucial in the activation of antigen-specific immune responses, such as naive T cells [65]. The field of cancer immunotherapy has been invigorated by the discovery that the vaccination with dendritic cells loaded with tumor antigens is a potent strategy to elicit protective immunity in tumor-bearing animal [66]. Dendritic cell therapy has improved much since it was first implemented, leading to an optimization that aims to induce a strong and broad immune response in terms of the recognized epitopes by both CD8$^+$ and CD4$^+$ T cells and the use of the patient's complete and unique set of the human leukocyte antigen, over the activation of humoral immunity [66,67].

This makes the choice of a particular tumor antigen with which to load the dendritic cells an important starting point in this type of therapy. The issues involved in this decision include the composition of the antigen, namely whether it is a defined tumor antigen or an unfractioned mixture of tumor-derived antigens; and the form in which the antigen should be presented, whether as a polypeptide or a nucleic acid [66]. The use of nucleic acid templates for the expression of tumor-derived antigens allows for the expression of the antigens as full-length proteins within dendritic cells. This, in turn, permits the patient's dendritic cells to display the peptides on their surface. On the other hand, vaccine strategies based on synthetic peptides or proteins require knowledge of the relevant peptides. Furthermore, the treatment is only suitable for a select group of patients with a matching human leucocyte agent type, often exclusively HLA-A2$^+$ [66,67].

Although DC-based cancer vaccines appear promising in terms of efficacy, many outstanding issues have been highlighted by recent trials, such as the need to define a standardized protocol and to minimize cost and time required for such treatments [65].

4. Future areas of study

As angiogenesis is a crucial part in the growth and maintenance of tumors [68], one of the major areas of research is the search for anti-angiogenesis, not only for oncology but also inflammatory and metabolic diseases where new vascularization is implicated in pathology. As previously stated, Cathepsin S offers an enticing target for such treatments.

Experiments have shown that the cartilage of rabbit and calf inhibits tumor angiogenesis. From those observations, attention has turned to cartilaginous fish, such as the shark, for a ready source of cartilage, hoping that it that may lead to a new answer in the inhibition of tumor angiogenesis [69]. Studies into the use of shark cartilage as a tumor anti-angiogenesis solution have not been conclusive [70], however, certain elements of the shark's adaptive immune system may provide possible avenues of treatment.

Sharks possess an antibody isotype, IgNAR, which is characterized as being a homodimer of IgH chains that do not use IgL chains. A domain sharing high identity to IgNAR was discovered at the amino terminus of T-cell Receptor δ [71]. This NAR-TCR variable domain is expressed in addition to the canonical TCRδV domain. Furthermore, the NAR-TCR δ chain contains two variable domains, both the product of V(D)J genetic rearrangement. This allows both B and T cells of cartilaginous fish to have the ability of making diverse repertoires of antigen receptors that recognize antigen with a projecting, free variable domain, as opposed to the more planar paratope of traditional heterodimeric receptors [69].

Contributing to the appeal of using shark IgNAR, is the ability to develop therapeutic binders that recognize epitope recesses and may not be accessible to the flatter paratope of IgH + IgL antibodies. Stability, tissue penetrance, B cell source, relatively simple single binding domain and the small size of shark IgNAR antibodies present attractive features that are being researched, both in academic and industrial laboratories worldwide, to be exploited by immunotherapy [69].

These antibodies have demonstrated potency which is equivalent or superior to that of their monspeficic IgG counerparts [8]. However, they still possess some clinical limitations that require further improvements, namely short half-lives and possible toxicity due to concurrent T cell co-stimulation [8,72].

A new format of bispecific antibodies are Bispecific T-cell engager (BiTE) molecules, which target both CD3 and another antigenic marker. These molecules show enhanced tumor cell lysis, high protein stability and efficacy at low T-cell/target ratios. BiTE antibodies may contribute to cancer immunotherapy by redirecting the vast number of existing T-cell clones in patients, while ignoring many of the immune escape mechanisms that otherwise limit the specific anti-tumor responses of T-cell clones [7].

Accumulating evidence suggests the existence of a subpopulation of tumor cells with distinct stem-like properties being responsible for tumor initiation, invasive growth and metastasis formation. Bispecific antibodies, capable of targeting both cancer stem cells as well as tumor antigens may prove to be effecting in eradicating said tumors, while limiting their reincidence [73]. A study reported the effectiveness of this therapy on cancer stem cells with a bispecific antibody against a variant form of the epidermal growth factor receptor (EGFRvIII) and CD133. Anti-EGFRvIII/CD133 mAb reduced the tumorigenicity of glioblastoma cells better than any reagent directed against a single epitope [74].

Researchers have also been looking into ways to alter the magnitude and quality of innate immune responses, induced by ADCC, in order to improve the ensuing adaptive immune response. The Fc receptors for IgG (FcγR) provide the key link between therapeutic antibodies and the cellular immune system, enabling monoclonal antibodies to induce adaptive immune responses. ADCC-based combination therapy can be used to promote antigen presentation, co-stimulation and T-cell activation or expansion. Antibody structures can also be modified to selective engage activating rather than inhibitory FcγR. The development of fusion antibodies also shows promise. Fusion antibodies possess immunostimulatory motifs, which can induce antigen presentation and amplify co-stimulation, resulting in a more efficient imune response [72,75–77].

5. Conclusion

It is quite clear that cancer immunotherapy has a bright future.

The amount of work and research that is being put into the discovery of new targets and treatments leaves us hopeful that a significant breakthrough might still be achieved during our lifetime. Immunotherapy presents opportunities to limit and eliminate tumor growth that simple chemotherapy has not been able to achieve. The extent and specificity of immunotherapy translate to quicker, better results without most of the undesirable side-effects.

While the number of available therapies keeps growing and the possibilities are truly exciting, it seems quite clear that the use of antibodies, especially monoclonal antibodies, is a truly promising path for the fight against cancer.

Monoclonal antibodies are supremely specific to their targets and have a relatively short lifespan inside the organism. This limits the undesirable side effects, while potentiating the anti-cancer capabilities of the therapy. Unfortunately, while this means that monoclonal antibody immunotherapy is considerably safer than other forms of anti-cancer therapy – namely small molecules – it is precisely due to their short lifespan that the efficacy of the treatment is limited.

This drawback might be overcome through the use of two simultaneous therapies, such as monoclonal antibodies paired up with chemotherapy. It is our hope that the shift might move away from the damaging effects of the latter on the patient; as it also attacks healthy cells, and onto the more pacific results of the former.

Immunotherapy as a medical research field continues to grow at an exciting pace, with new options and hypothesis being introduced as cancer biology becomes better understood. It is for these reasons that we look forward to more advances in the field, as well as to providing our own contribution in the fight against cancer.

Conflict of interest disclosure

The authors declare no competing financial interests.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgments

References

[1] B. Fauvel, A. Yasri, Antibodies directed against receptor tyrosine kinases: current and future strategies to fight cancer, mAbs 6 (4) (2014) 10–11.

[2] M. Stern, R. Herrman, Overview of monoclonal antibodies in cancer therapy: present and promise, Crit. Rev. Oncol. Hematol. 56 (1) (2005) 11–59.

[3] L.M. Nadler, et al., Serotherapy of a patient with a monoclonal antibody directed against a human lymphoma-associated antigen, Cancer Res. 40 (1980) 3147–3154.

[4] R.M. Sharkey, D.M. Goldenberg, Targeted therapy of cancer: new prospects for antibodies and immunoconjugates, CA Cancer J. Clin. 56 (2006) 226–243.

[5] B. Kahl, Chemotherapy combinations with monoclonal antibodies in non-Hodgkin's lymphoma, Semin. Hematol. 45 (2) (2008) 90–94.

[6] S.A. Rosenberg, et al., Cancer immunotherapy: moving beyond current vaccines, Nat. Med. 45 (2) (2004) 909–915.

[7] P.A. Baeuerle, C. Reindhardt, Bispecific T-cell engaging antibodies for cancer therapy, Cancer Res. 69 (2009) 4941.

[8] L.S. Shahied, et al., Bispecific minibodies targeting HER2/neu and CD16 exhibit improved tumor lysis when placed in a divalent tumor antigen binding format, J. Biol. Chem. 279 (2004) 53907–53914.

[9] P. Chames, et al., Therapeutic antibodies: successes, limitations and hopes for the future, Br. J. Pharmacol. 157 (2) (2009) 220–233.

[10] R.K. Oldham, R.O. Dillman, Monoclonal antibodies in cancer therapy: 25 years of progress, J. Clin. Oncol. 26 (11) (2008) 1774–1777.

[11] C.J. Tape, et al., Cross-domain inhibition of TACE ectodomain, PNAS 108 (14) (2011).

[12] M.L. Moss, et al., Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-alpha, Nature 385 (1997) 733–736.

[13] R.A. Black, et al., A metalloproteinase disintegrin that releases tumour-necrosis, Nature 385 (1997) 729–733.

[14] S.W. Sunnarborg, et al., Tumor necrosis factor-alpha converting enzyme (TACE) regulates epidermal growth factor receptor ligand availability, J. Biol. Chem. 277 (2002) 12838–12845.

[15] C. Brou, et al., A novel proteolytic cleavage involved in Notch signalling: the role of the disintegrin-metalloprotease TACE, Mol. Cell 5 (2000) 207–216.

[16] T.P. Condon, et al., ADAM17 but not ADAM 10 mediates tumor necrosis factor-alpha and L-selectin shedding from leukocyte membranes, Antisense Nucleic Acid Drug Dev. 11 (2001) 107–116.

[17] V. Marin, et al., Chemotactic agents induce IL-6Ralpha shedding from polymorphonuclear cells: involvement of a metalloproteinase of the TNF-alpha-converting enzyme (TACE) type, Eur. J. Immunol. 32 (10) (2002) 2965–2970.

[18] P.A. Kenny, TACE: a new target in epidermal growth factor receptor dependent tumors, Differentiation 75 (2007) 800–808.

[19] M.L. Moss, L. Sklair-Tavron, R. Nudelman, Drug insight: tumor necrosis factor-converting enzyme as a pharmaceutical target for rheumatoid arthritis, Nat. Clin. Pract. Rheumatol. 4 (2008) 300–309.

[20] P. Saftig, K. Reiss, The "A Disintegrin And Metalloproteases" ADAM10 and ADAM17: novel drug targets with therapeutic potential? Eur. J. Cell Biol. 90 (2011) 527–535.

[21] F. Richards, et al., Anti-tumour effects of a specific anti-ADAM17 antibody in an ovarian cancer model in vivo, PLoS ONE 7 (7) (2012) e40597.

[22] H.F. Kwok, et al., Development of a 'mouse and human cross-reactive' affinity-matured exosite inhibitory human antibody specific to TACE (ADAM17) for cancer immunotherapy, Protein Eng. Des. Sel. 27 (6) (2014) 1–12.

[23] J. Bostrom, et al., Variants of the antibody herceptin that interact with HER2 and VEGF at the antigen binding site, Science 323 (2009) 1610–1614.

[24] J. Gao, S.S. Sidhu, J.A. Wells, Two-state selection of conformation-specific antibodies, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 3071–3076.

[25] X. Hu, et al., Combinatorial libraries against libraries for selecting neoepitope activation-specific antibodies, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 6252–6257.

[26] S. Willems, et al., Thiol isomerases negatively regulate the cellular shedding activity of ADAM17, Biochem. J. 428 (2010) 439–450.

[27] D.R. Edwards, M.M. Handsley, C.J. Pennington, The ADAM metalloproteinases, Mol. Asp. Med. 29 (5) (2008) 258–289.

[28] P.A. Kenny, M.J. Bissell, Targeting TACE-dependent EGFR ligand shedding in breast cancer, J. Clin. Invest. 117 (2) (2007) 337–345.

[29] R. Burden, Antibody targeting of Cathepsin S induces antibody-dependent cellular cytoxicity, Mol. Cancer 10 (2011) 147.

[30] H.F. Kwok, Antibody research targeting Cathepsin S for cancer therapy, Adv. Biosci. Biotechnol. 4 (2013) 17–20.

[31] P. Verderio, Synthesis and Biofunctionalization of Novel Composite Nanocarriers for Targeted Detection and Treatment of Malignant Cells(PhD thesis) 2014.

[32] X. X., et al., Identification of a new epitope in uPAR as a target for the cancer therapeutic monoclonal antibody ATN-658, a structural homolog of the uPAR binding integrin CD11b (αM), PLoS ONE 9 (1) (2014) e85349.

[33] R.E. Burden, et al., Antibody-mediated inhibition of Cathepsin S blocks colorectal tumor invasion and angiogenesis, Clin. Cancer Res. 15 (2009).

[34] D.G. Maloney, et al., DEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma, BloodSep 90 (6) (1997) 2188–2195.

[35] M. Li, et al., Nanoscale distribution of Cd20 on B-cell lymphoma tumour cells and its potential role in the clinical efficacy of Rituximab[J], J. Microsc. 254 (1) (2014) 19–30.

[36] M.J. Leandro, B-cell subpopulations in humans and their differential susceptibility to depletion with anti-CD20 monoclonal antibodies, Arthritis Res. Ther. 15 (Supplement 1) (2013) S3.

[37] L.E. Van Der Kolk, et al., Complement activation plays a key role in the side-effects of rituximab treatment, Br. J. Haematol. 115 (2001) 807–811.

[38] K.M. King, A. Younes, Rituximab: review and clinical applications focusing on non-Hodgkin's lymphoma, Expert. Rev. Anticancer. Ther. 1 (2) (2001) 177–186.

[39] J.C. Byrd, et al., Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance, JCO 17 (3) (1999) 791.

[40] H.M. Gürcan, et al., A review of the current use of rituximab in autoimmune diseases, Int. Immunopharmacol. 9 (2009) 10–25.

[41] R. Eisenberg, Update on Rituximab, Ann. Rheum. Dis. 64 (Supplement 4) (2005) 55–57.

[42] B.A.C. Cree, et al., An open label study of the effects of rituximab in neuromyelitis optica, Neurology 64 (7) (2005) 1270–1272.

[43] B. Friedrichs, et al., The effects of rituximab treatment during pregnancy on a neonate, Haematologica 91 (2006) 1426–1427.

[44] J. Golay, et al., Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis, Blood 95 (12) (2000) 3900–3908.

[45] R. Nahta, F.J. Esteva, Herceptin: mechanisms of action and resistance, Cancer Lett. 232 (2006) 123–138.

[46] S.D. Cosimo, J. Baselga, Targeted therapies in breast cancer: where are we now? Eur. J. Cancer 44 (2008) 2781–2790.

[47] A.M. Scott, et al., Antibody therapy of cancer, Nat. Rev. Cancer 12 (2012) 278–287.

[48] D.J. Slamon, et al., Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N. Engl. J. Med. 344 (2001) 783–792.

[49] M. Martín, et al., Minimizing cardiotoxicity while optimizing treatment efficacy with trastuzumab: review and expert recommendations, Oncologist 14 (2009) 1–11.

[50] W. Dean-Colomb, F.J. Esteva, Her2-positive breast cancer: Herceptin and beyond, Eur. J. Cancer 44 (2008) 2806–2812.

[51] K.A. Gelmon, et al., Use of trastuzumab beyond disease progression: observations from a retrospective review of case histories, Clin. Breast Cancer 5 (1) (2004) 52–58.

[52] S.L. Topalian, et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, N. Engl. J. Med. (2012) 2443–2454.

[53] C.G. Drake, et al., Safety, Durable Clinical Benefit, and Remission Resulting from Nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in a Phase 1 Trial in Patients with Previously Treated Metastatic Renal Cell Carcinoma (mRCC); Long-term Patient Follow-up, 2014.

[54] M.A. Postwo, et al., Peripheral and tumor immune correlates in patients with advanced melanoma treated with nivolumab (anti-PD-1, BMS-936558, ONO-4538) monotherapy or in combination with ipilimumab, J. Transl. Med. 12 (1) (2014) O8.

[55] K. Ofuji, et al., Critical analysis of the potential of targeting GPC3 in hepatocellular carcinoma, J. Hepatocellular Carcinoma 1 (2014) 35–42.

[56] P.J. Morin, Claudin proteins in human cancer: promising new targets for diagnosis and therapy, Cancer Res. 65 (21) (2005) 9603–9606.

[57] S.K. Kumar, et al., Improved survival in multiple myeloma and the impact of novel therapies, Blood 111 (5) (2008) 2516–2520.

[58] Y. Tai, et al., Novel anti-B-cell maturation antigen antibody–drug conjugate (GSK2857916) selectively induces killing of multiple myeloma, Blood 123 (20) (2014) 3128–3138.

[59] L. Gattinoni, et al., Adoptive immunotherapy for cancer: building on success, Nat. Rev. Immunol. 6 (5) (2006) 383–393.

[60] D. Chung and e. al., "A new hope in immunotherapy for malignant gliomas: adoptive T cell transfer therapy," Journal of Immunology Research, vol. Not yet published, 2014.

[61] S.R. Mattarollo, et al., Chemotherapy pretreatment sensitizes solid tumor-derived cell lines to Vα24 + NKT cell-mediated cytotoxicity, Int. J. Cancer 119 (7) (2006) 147–153.

[62] T. Kim, et al., Immunological factors relating to the antitumor effect of temozolomide chemoimmunotherapy in a murine glioma model, Clin. Vaccine Immunol. 17 (1) (2010) 147–153.

[63] C.E. Fadul, et al., Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination after radiation chemotherapy, J. Immunother. 34 (4) (2011) 382–389.

[64] T.O. Kabilova, et al., Immunotherapy of hepatocellular carcinoma with small double-stranded RNA, BMC Cancer 14 (338) (2014).

[65] S. Pejawar-Gaddy, O.J. Finn, Cancer vaccines: accomplishments and challenges, Crit. Rev. Oncol. Hematol. 67 (2) (2008) 93–102.

[66] D.A. Mitchell, S.K. Nair, RNA-transfected dendritic cells in cancer immunotherapy, J. Clin. Invest. 106 (9) (2000) 1065–1069.

[67] A.M.T. Van Nuffel, et al., Loading of dendritic cells for immunotherapy, ISBT Sci. Ser. Int. J. Intracell. Transp. 8 (1) (2013) 161–164.

[68] J. Folkman, What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst. 82 (1) (1990) 4–7.

[69] M.F. Criscitiello, What the Shark Immune System Can and Cannot Provide for the Expanding Design Landscape of Immunotherapy, 2014. ([Online]).

[70] L. C.L., et al., Evaluation of shark cartilage in patients with advanced cancer: a north central cancer treatment group trial, Cancer 104 (1) (2005) 176–182.

[71] C. M.F., et al., An evolutionarily mobile antigen receptor variable region gene: doubly rearranging NAR-TcR genes in sharks, Proc. Natl. Acad. Sci. U. S. A. 103 (13) (2006) 5036–5041.

[72] L.M. Weiner, et al., Monoclonal antibodies: versatile platforms for cancer immunotherapy, Nat. Rev. Immunol. 10 (2010) 317–327.

[73] M.P. Deonarain, et al., Antibodies targeting cancer stem cells, mAbs 1 (1) (2009) 12–26.

[74] L. Gammaitoni and e. al., "Immunotherapy of cancer stem cells in solid tumors: initial findings and future prospective," Expert Opin. Biol. Ther., vol. Not yet published, 2014.

[75] K.D. Khan, et al., A phase 2 study of rituximab in combination with recombinant interleukin-2 for rituximab-refractory indolent non-Hodgkin's lymphoma, Clin. Cancer Res. 12 (2006) 7046–7053.

[76] L.S. Metelitsa, et al., Antidisialoganglioside/granulocyte macrophage-colony-stimulating factor fusion protein facilitates neutrophil antibody dependent cellular cytotoxicity and depends on FcγRII (CD32) and Mac-1 (CD11b/CD18) for enhanced effector cell adhesion and azurophil gra, Blood 99 (2002) 4166–4173.

[77] B. Jahrsdorfer, G.J. Weiner, Immunostimulatory CpG oligodeoxynucleotides and antibody therapy of cancer, Semin. Oncol. 30 (2008) 476–482.