With the widespread use of therapeutic monoclonal antibodies in the treatment of patients with cancer, resistance to these agents has become a major issue. Preclinical models of drug action or resistance have contributed to unravel the main mechanisms of resistance, involving both tumor-associated and host related factors. However our understanding of how a monoclonal antibody destroys cancer cells in a patient and why it one day stops being effective are still far from being complete. This review focuses on the available data on mechanisms of action and resistance to rituximab and includes some additional information for other monoclonal antibodies. Innovative approaches designed to overcome resistance, such as combination immunotherapy, costimulation with cytokines or growth factors are presented.

Introduction

Passive immunotherapy of malignancies with therapeutic monoclonal antibodies (mAbs) has profoundly modified the way clinicians consider treatment of patients afflicted with haematological malignancies or solid tumors. While some patients can benefit from mAbs administered as single agent first line therapy and/or as consolidation therapy, most patients receiving therapeutic mAbs will do so in the scope of combinations with classical antimitotic compounds, or, in the near future, with small molecule targeted therapies.

While it is clear that mAbs have significantly contributed to improving the outcome of patients afflicted with cancer, there is no proof that mAbs have modified the curability of those types of cancer which could not be cured by conventional treatments. In the case of lymphoma patients for example, the combination of rituximab with the CHOP regimen (cyclophosphamide, hydroxydaunomycin, Oncovin, prednisone) has demonstrated improved response rates, freedom from progression and overall survival in patients with diffuse large B cell lymphoma (NHL), a subtype which could in some patients be cured by CHOP alone. Conversely in patients afflicted with follicular lymphoma (FL), an indolent yet incurable disease, rituximab has profoundly modified the way patients are treated, but does not appear to have made the disease curable. Approximately 50% of patients with relapsed/refractory CD20+ follicular lymphomas do not respond to initial therapy with rituximab and close to 60% of prior rituximab responding patients will not longer benefit with retreatment with this monoclonal antibody. Likewise patients with solid tumors who were considered incurable with conventional therapy have not presently been shown to be cured by the addition of mAbs.

Whether administered as single agents or in combination regimens, the therapeutic activity of mAbs is therefore limited by mechanisms of resistance. Whether these forms of resistance are innate or acquired, there is an urgent need to better understand why tumor cells are resistant or how they become resistant to mAbs, and which strategies could be implemented to circumvent these resistance mechanisms in patients.

Resistance to cancer therapy has mainly been explored for systemic treatments such as chemotherapy, and been designated under the term of chemoresistance. While chemoresistance was initially observed after the first unsuccessful attempts to treat leukemia patients with nucleotide analogues fifty years ago, the history of chemoresistance really starts with the discovery of the P glycoprotein efflux protein by Ling et al. in the 1970s. Lessons learned while trying to understand and circumvent the function of proteins such as P glycoprotein remain of great use in the study of newer agents, both in terms of understanding preclinical (most notably pharmacokinetics) and cellular (pharmacodynamics)
Understanding and circumventing resistance to anticancer monoclonal antibodies

Resistance mechanisms. Along the same line, the large amount of data accumulated regarding resistance mechanisms to classical anticancer agents are also useful in understanding resistance to mAbs, insofar as the classical agents and mAbs share similar apoptotic effector mechanisms.

Antibodies often exhibit complicated pharmacokinetic and pharmacodynamic properties.\(^5\) Due to the multiple mechanisms of antibody cytotoxicity and the complex nature of the antibody disposition, the determination of these parameters will lead to improved development of monoclonal antibodies.

mAbs are similar to conventional agents in that they undergo degradation and clearance and induce apoptotic signaling, however, they differ by the fact that factors independent of the tumor cell itself strongly contribute to their anticancer effect. Complement Dependent Cytotoxicity (CDC) and Antibody Dependent Cellular Cytotoxicity (ADCC) are considered to be essential mechanisms of action of antitumor activity of mAbs, and are therefore likely to be involved in the development of resistance mechanisms. In this review, we will discuss available data regarding preclinical models of resistance to mAbs, focusing on rituximab, as well as results correlated with response to mAbs in the clinic. These data have shed some light on potential mechanisms of resistance to therapeutic mAbs, and suggest possible strategies to circumvent these resistance phenomena.

Rituximab

In 1997, rituximab became the first monoclonal antibody approved for cancer therapy.\(^6\) Having been used for over a decade in patients, rituximab is thus the therapeutic mAb for which there are currently the most data, both in terms of mechanisms of action, parameters associated with sensitivity or resistance, and strategies to enhance its antitumor effect. Rituximab is a chimeric anti CD20 monoclonal antibody composed of murine variable regions (Fab region) that are linked to a human Fc component, targeting the CD20 antigen. CD20 antigen is a transmembrane protein of 35 kD molecular weight, located mainly in pre-B and mature B lymphocytes but not on stem or plasma cells. Its role is still unclear, but there is evidence that it may be involved in regulating cell cycle and differentiation processes, and could behave as a calcium ion channel as well.\(^7\)

Models used to Understand Rituximab Cytotoxicity or Resistance to Rituximab

Preclinical models of rituximab are illustrative of the difficulties involved in identifying resistance mechanisms to mAbs. As for most unlabelled mAbs, rituximab demonstrates poor cytotoxic effect per se on cell lines expressing the target antigen in vitro, and is much more effective when CDC or ADCC are reproduced in the test tube by the addition of fresh human serum and/or peripheral blood effector cells, respectively. Induction of apoptosis by rituximab alone has been reported in the absence of accessory cells, but has mostly been described using cell lines derived from patients with Burkitt lymphoma, a subtype of NHL for which the clinical indication of rituximab has not yet been as well documented.\(^8,9\)

Conversely rituximab has been shown by several groups to possess activity in murine models of xenotransplanted human CD20 positive lymphoma lines. Notwithstanding the limitations due to the use of immunocompromised mice, these models have been very informative in determining the contribution of CDC or ADCC in vivo, and offer the possibility of analyzing signaling pathways in tumors. Experiments with cobra venom factor, a complement-depleting agent, have shown that the antitumor effect of rituximab is at least partly CDC-dependent in vivo.\(^10-12\) Other experiments involving the depletion of NK cells, macrophages or granulocytes have been performed, sometimes with contradictory results, but overall suggest an important role for ADCC in rituximab cytotoxic activity.\(^13\) Conversely there are currently few data available regarding apoptotic signalization in in vivo samples.

Clinical samples have been used to better understand how rituximab works using different approaches. In the “ex vivo” approach, fresh human samples, most commonly peripheral blood containing malignant cells, are exposed to rituximab and cell death can then be quantified.\(^14\) These models are interesting insofar as the samples have not been altered by prolonged growth in vitro, and that autologous effector factors (patient serum and/or accessory cells) can be used. However, these studies are difficult to generalize to patients with solid tumors for obvious reasons. Even in the context of haematological malignancies one must keep in mind the differences occurring within blood, bone marrow, lymph nodes and other tissues. Clearance of malignant cells from the blood is known to be more readily obtained than that of bone marrow or lymph nodes, suggesting that the study of blood samples might not be representative of other tissues. Clinical samples have also been used to establish correlations between the genetic makeup of the patient and response to rituximab using normal cells to study genetic polymorphisms.\(^15,16\) Tumor samples may also be used to analyse expression profiles and establish correlations with response to mAbs.\(^17\)

Parameters Correlated with Rituximab Activity and Resistance

The mechanisms that influence rituximab efficiency include host and tumor cell-related factors. Host-related factors that possibly have an impact on rituximab are diverse, ranging from pharmacokinetic parameters to accessory effector mechanisms and intracellular signaling pathways (Fig. 1). Little is currently known regarding the pharmacokinetics of rituximab, although clinical studies have shown a large interindividual variability in rituximab exposure and its significant influence on clinical response in patients receiving similar doses of antibody.\(^18,19\) Dayde et al. have shown in a preclinical model that exposure to rituximab influences response and survival.\(^20\) Additional investigations are clearly warranted to better define parameters influencing pharmacokinetic parameters of rituximab.

Individual variations in accessory mechanisms are also likely to influence the cytotoxic activity of rituximab. ADCC relies on the binding of the Fc portion of rituximab to Fc γ receptors on accessory cells. The relative ratio of “activating” receptors such as FcγRI, FcγRIIA, FcγRIII and “inhibitory” receptors...
such as FcγRIIB is likely to determine the net interaction with accessory cells after rituximab binding. Cartron et al. analyzed the impact of the FCGR3A-158V/F polymorphism by genotyping 48 patients having received single agent rituximab as first line therapy for FL. The objective response rates at 12 months was 90% in FCGR3A-158V homozygous patients and 51% in FCGR3A-158F carriers ($p = 0.03$). In murine models depletion of accessory cells such as macrophages (using liposomal clodronate) or NK cells (using specific mAbs) has been shown to reduce the cytotoxic activity of rituximab. These data globally support the role of ADCC as a clinically relevant effector mechanism of rituximab in vivo.

Complement-dependent cytotoxicity is also likely to vary from one patient to another. Golay et al. investigated the role of the complement inhibitors CD35, CD46, CD55 and CD59 with blocking antibodies in FL cell lines as well as in fresh cases of FL and showed that CD55, and to a lesser extent CD59, were important regulators of complement-mediated cytotoxicity. These observations were further supported by the results of Treon et al. who found that anti-CD59 mAbs sensitized cells to rituximab cytotoxicity, and of Takei et al. who observed increased expression of CD55 and CD59 in rituximab-resistant Ramos cells. More recently Racila et al. genotyped the C1qA([276A/G]) polymorphism in 133 subjects with FL treated with single-agent rituximab and observed a significantly different time to progression in homozygous G subjects (282 days) and in A-allele carriers (708 days, $p = 0.02$). Homozygous A subjects achieved complete response at a higher rate than heterozygous or homozygous G subjects.

Tumor-related factors that are involved in resistance to rituximab include alteration in CD20 and lipids raft domain and regulation in signaling and mitochondrial pathways (Fig. 2). Alterations of the CD20 antigen are prime suspects as causes of resistance to rituximab. However, there are very few data in the literature regarding CD20 mutations.
and little more regarding correlations between CD20 expression and sensitivity to rituximab. Terui et al. sequenced the CD20 gene in samples from 68 NHL patients receiving rituximab and found mutations in 12 patients. These authors reported a lower CD20 expression level in patients bearing a mutation in the C-terminal cytoplasmic domain. Reduced CD20 expression has been reported by several authors in cell lines rendered resistant to rituximab in vitro but have only anecdotally been reported in patients relapsing after rituximab. An in vitro Burkitt model resistant to rituximab developed by Jazirehi et al. has shown a 50% reduction of CD20 expression in resistant clones, and this was confirmed in another in vitro model of follicular lymphoma. However, in our in vivo model of follicular lymphoma using the human RL line resistant to rituximab, CD20 expression was not different in the resistant cells in comparison to the sensitive parental cells. Interestingly, there appears to be a correlation between the baseline level of expression of CD20 in various subtypes of lymphoproliferative diseases and clinical responsiveness to rituximab. Chronic lymphocytic leukemia (CLL) cells tend to have low expression of CD20, as opposed to marginal zone lymphoma (MZL) or DLCL for example. Quantification of CD20 is however difficult to perform reliably, and flow cytometry has been reported to be more precise than immunohistochemistry.

After interaction with rituximab, CD20 has been shown to be redistributed to rafts, or detergent-insoluble microdomains. This appears to be a common finding for type I antibodies, but is not observed with type II antibodies such as tositumomab or GA101. Raft components and/or factors affecting redistribution of CD20 to rafts may impact on the activity of rituximab. Meyer zum Buschenfelde et al. have recently reported that the content in GM1, a raft-associated sphingolipid, in patient samples was correlated with sensitivity to rituximab. Samples from patients with MZL, a subtype sensitive to rituximab, were found to have high GM1 content, while CLL samples had a lower GM1 content. Deficient redistribution into rafts or alterations in the composition of rafts are thus likely mechanisms of resistance to rituximab, although this remains to be studied in greater detail. The fact that type II antibodies do not appear to require redistribution to rafts suggest that they may be active in models of resistance to rituximab.

Rituximab binding has been shown to activate a number of signalization pathways, either inducing cell death or sensitizing tumor cells to cytotoxic agents. The Bonavida group has shown thatraf kinase inhibitor protein plays a key role in regulating Bcl-xL, through NFKappaB and MAPkinase pathways. Other antiapoptotic genes, such as Bfl1, or proapoptotic genes, such as Bax or Bak, have also been found to influence sensitivity to rituximab. More recently it has also been found that Yin Yang and PKC (ζ) were involved in rituximab signaling. Suzuki et al. recently suggested that rituximab might suppress the constitutively active Akt pathway in NHL cells, without modifying unphosphorylated Akt levels. The clinical relevance of apoptotic signalization as compared to that of extracellular mechanisms such as CDC and ADCC is difficult to determine. Whether apoptotic induction by rituximab per se occurs or not in vivo, it is highly likely that CD20-mediated signalization sensitizes NHL cells to the cytotoxic activity of conventional chemotherapeutic agents.

Both caspase-dependent and caspase-independent cell death have been reported after exposure to rituximab. Byrd et al. reported activation of caspase-9, caspase-3 and poly(ADP-ribose) polymerase (PARP) cleavage as well as significant down-modulation of the antiapoptotic proteins XIAP and Mcl-1 in CLL patients receiving rituximab treatment. More recently Stolz reported that rituximab triggers apoptosis through mitochondrial-mediated caspase pathways. Conversely caspase-independent toxicity has also been described by various authors, and may involve the role of calcium.

Several studies have shown that resistant cells display constitutive hyperactivation of the survival pathways NFkB and ERK1/2, leading to overexpression of Bcl-2, Bcl-2-related gene and Mcl-1. In the in vivo resistant RL model, Bcl-XL was also found more highly expressed in rituximab-resistant cells. This confirms the recent results obtained in vitro by Jazirehi et al. showing that the phenotype of resistant cells to rituximab may be associated with a higher expression of Bcl-XL. Moreover, we found an overexpression of YY1, a negative regulator of Fas and Trail receptor DR5 expression, that can inhibit apoptosis. Altered signaling pathways have been also shown to be associated with a downregulation of the pro-apoptotic Bcl2 family proteins BAX and BAK responsible for associated resistance to chemotherapy, thereby blocking initiation of apoptosis. A low ratio of Bax (or Bax) to Bcl-2 in tumor cells was associated with increased survival in patients with follicular lymphoma while a low ratio of Bax to Mcl-1 was associated with resistance to rituximab in chronic lymphocytic leukemia patients. These data therefore suggest that Bcl2 family proteins, involved in the regulation of apoptosis, and well-known as being involved in the sensitivity to antimitotic compounds, are also likely to be clinically relevant in terms of sensitivity to anticancer mAbs.

Cetuximab

Cetuximab is a monoclonal chimeric antibody directed against the epidermal growth factor receptor (EGFR). EGFR is overexpressed in a variety of solid tumors, suggesting an important role in the process of neoplastic transformation. Cetuximab binds to EGFR with a 2-log higher affinity than the natural ligands TGFα and EGF. Therefore, its binding deactivates many cellular pathways such as the mitogen-activated protein kinase, phosphatidylinositol 3 kinase and Akt pathways. When competing with receptor binding, cetuximab induces receptor internalization and prevents ligand-mediated receptor tyrosine kinase phosphorylation. It may also exert its anti-tumor effects through ADCC via its fragment c receptor (FCR). Two polymorphisms FCGR2A-H131R and FCGR3A-V158F were independently associated with progression-free survival and may be useful as molecular markers to predict clinical outcome in metastatic CRC patients treated with cetuximab.

It has recently been shown that patients with advanced colorectal cancer do not respond to anti-EGFR therapies such as panitumumab and cetuximab if tumors contain KRAS mutations. KRAS status was found to be an independent prog nostic...
Understanding and circumventing resistance to anticancer monoclonal antibodies

factor associated with overall survival and progression free survival. Testing for KRAS mutations is fast becoming a clinically relevant predictor for patients whose disease justifies treatment with cetuximab. A BRAF V600E mutation was also detected in some patients who did not respond to neither cetuximab nor panitumumab and could be a useful biomarker for selecting patients responsive to anti-EGFR therapy.53 Thus, combination therapy which can block both EGFR and BRAF in patients with BRAF-mutated tumours may be an effective therapy in non-responder patients. Other parameters, including PIK3CA mutation/PTEN expression status54 or specific gene expression profiles, have also been suggested to influence response to cetuximab.55

Models used to Understand Cytotoxicity of Cetuximab

To understand the molecular mechanisms of acquired resistance to EGFR inhibitors, Wheeler et al.56 established a series of cetuximab-resistant clones in vitro following long-term exposure to cetuximab in nonsmall cell lung cancer (NSCLC; H226) and head and neck squamous cell carcinoma (HNSCC; SCC-1) cell lines. These authors report that cetuximab-resistant cells show altered EGFR internalization and degradation as well as enhanced expression of HER2, HER3 and c-Met. Benavente et al.57 presented recently another model of resistance to cetuximab, gefitinib or erlotinib in head and neck tumor cells following chronic exposure to these agents. EGFR inhibitor-resistant lines showed increased proliferation rates and elevated levels of phosphorylated EGFR, MAPK, AKT and STAT 3, with reduced apoptotic capacity. These important observations raise the possibility that combined targeting of these pathways, using other mAbs or small molecule inhibitors of downstream pathways may enhance the antitumor activity of cetuximab.

Trastuzumab

Trastuzumab is a recombinant humanized monoclonal antibody which binds to the IV domain of the extracellular segment of HER2. The HER2 protein is involved in the regulation of normal breast growth and development.58,59 HER2 gene amplification/protein overexpression has been detected in 20 to 30% of human breast carcinomas and studies have indicated that HER2 amplification/overexpression plays a role in malignant transformation and tumorigenesis.60 Cells treated with trastuzumab undergo arrest during the G1 phase of the cell cycle, downregulate HER2 leading to disruption of receptor dimerization and signaling through the downstream PI3K and MAP (MAPK) cascades. The efficacy of trastuzumab may also depend upon its ability to induce an immune response. It can promote apoptosis in multiple breast cancer lines via antibody-dependent cellular cytotoxicity (ADCC).61 Musilino et al. showed that FcγR polymorphisms play a role in trastuzumab-mediated ADCC and may be a predictor tool for clinical outcome of patients with breast cancer treated with trastuzumab-based therapy. ADCC could therefore be an additional mechanism in the response to trastuzumab that is particularly effective in patients who are FcγR1B158V and/or FcγRlla 131H homozygous.62

Several mechanisms of resistance to trastuzumab have been reported. The overexpression of MUC4, a membrane-associated glycoprotein, can sterically hinder the antibody from binding HER2 surface receptor and may mediate a crosstalk to activate HER2, leading to tumor progression and metastasis.63,64 In breast cancer cell models that overexpress HER2/neu, Lu et al. showed that an increased level of IGF-1R signaling appeared to interfere with the action of trastuzumab.65 Furthermore, the Met receptor tyrosine kinase has also been reported to contribute to trastuzumab resistance.66 These data suggest that a variety of cell surface receptors, other than HER2, and/or its downstream signaling proteins are likely to influence sensitivity to trastuzumab. Comparing the sensitivity of 18 breast cancer lines to trastuzumab, Ginestier et al. found that sensitivity to trastuzumab was frequently associated with the expression of a phosphorylated ERBB2 protein.67 Another potential mechanism of resistance is the accumulation of truncated forms of the HER2 receptor that lack the extracellular trastuzumab-binding domain. This form, known as p95HER2, is frequently found in HER2-expressing breast cancer cell lines and tumors. Scaltriti et al.68 demonstrated that cells that expressed p95HER2 were resistant to trastuzumab, but remained sensitive to lapatinib both in vitro and in vivo.

Regarding intracellular signaling, various reports suggest that alterations in specific pathways can be associated with resistance to trastuzumab. A loss of RALT/MIG-6, a transcriptionally controlled feedback inhibitor of ErbB receptor tyrosine kinases, was found to favor resistance to trastuzumab.59 T-DARPP, a protein associated with ERBB2, has been shown to regulate sensitivity to trastuzumab in preclinical breast cancer models.70 In a cohort of 55 breast cancer patients, activation of the PI3K pathway, as judged by the presence of oncogenic PIK3CA mutations or low PTEN expression, was associated with poor prognosis after trastuzumab therapy.71 Interestingly these factors are similar to those identified by a genome wide scan of factors involved in resistance to lapatinib, a small molecule inhibitor of HER2 tyrosine kinase.72 These data also confirm previous results showing that PTEN is involved in sensitivity to trastuzumab.73

Strategies to Circumvent Resistance to Monoclonal Antibodies

Current data suggest that resistance to therapeutic mAbs is multifactorial and is likely to involve, among other parameters, host-related effector mechanisms, altered interaction with the target, cross-talk between cell survival pathways and involvement of antiapoptotic proteins. It is highly likely that most resistance events downstream of the interaction with the target antigen will be redundant with those observed with small molecule tyrosine kinase inhibitors, and that several will be similar to those already reported with cytotoxic agents. Insofar as therapeutic mAbs will most commonly be used in combination regimens, avoiding or overcoming resistance will thus involve the simultaneous targeting of non-redundant death-inducing pathways, or the neutralization of compensatory mechanisms.

Several strategies have been proposed to increase rituximab activity or to revert resistance to rituximab. An elegant approach has consisted in the physical costimulation of CD20 and another cell surface antigen, either with a multivalent mAb or with a
recombinant protein. Fas, CD22 and TRAIL have thus been shown to be potential co-targets of CD20.74,75 Simultaneous targeting of two antigens with two antibodies is also an option and rituximab combined with epratuzumab, a CD22-directed antibody, demonstrated promising antilymphoma activity in a study conducted in patients with recurrent or refractory non-Hodgkin lymphoma.76 Preclinical as well as clinical data suggest that simultaneous targeting of CD20 with rituximab and CD52 with alemtuzumab could also constitute a way to enhance antilymphoma activity.27,77,78 Another possibility is to potentiate cellular effector mechanisms using cytokines or growth factors. The feasibility of this approach, using GM-CSF, has recently been reported.79 Other studies evaluated the combination of rituximab with interferon-α (INFα),80,81 interleukin-12 (IL-12),82 IL-2,83 in order to enhance effector immune cells. Further elucidation of multiple mechanisms of action and critical signaling pathways involved in rituximab cytotoxicity will help to overcome resistance.

Novel MAbs are currently undergoing pre-clinical and clinical investigation. GA101 is a fully humanized anti-CD20 with a glyco-engineered Fc portion and a modified elbow hinge. Its glycoengineered Fc region binds with 50-fold higher affinity to human FcγRIII receptors compared to a standard, non-glycoengineered antibody such as rituximab. This modification has led to complete responses and long-term survival in xenograft models of diffuse large B cell lymphoma and mantle cell lymphoma84 and has been shown to be more active than rituximab on RL xenografts at similar doses, either administered as a single agent or in combination with cyclophosphamide.85

Novel therapeutic strategies are underway to improve response rates in HER2-overexpressing and in trastuzumab-refractory patients. Pertuzumab, belonging to a new class called dimerization inhibitors that can inhibit signaling by other HER family receptors, as well as inhibiting signaling in cells that express normal level of HER2. It can also disrupt interaction between HER2 and IGF-IR in trastuzumab-resistant cells.86 Recently, studies have suggested that the dual HER2/EGFR tyrosine kinase inhibitor lapatinib targeted against both EGFR and HER2 inhibited the growth of HER2-overexpression breast cancer cells in patients receiving prolonged treatment with trastuzumab,87 and inhibited insulin-like growth factor I (IGF-I) signaling in resistant cells.88 This type of approach has potential in HER2-overexpression breast cancers, as well as in trastuzumab-refractory patients, and constitutes a novel strategy that cotargets the IGF-I receptor and HER2 pathways. Along the same line, novel IGF-IR targeted agents and PI3K inhibitors are currently studied for potential use in trastuzumab refractory patients.

New strategies have also been developed to optimize the therapeutic effects of EGFR inhibitors, by exploring new EGFR-targeted mAbs such as panitumumab,89 or matuzumab.90 Several clinical studies are ongoing to evaluate the combination of cetuximab with bevacizumab (anti-VEGF Mab) after the encouraging preliminary results of Saliz et al.91 Another approach involves the association of cetuximab with small molecule tyrosine kinase inhibitors (TKIs) such as erlotinib, gefitinib92 and lapatinib,94 This combined approach appears to be an effective strategy to increase the inhibition of EGFR autophosphorylation, cellular proliferation and downregulation of signaling pathways. However, the development of acquired resistance in treated patients reduces the efficiency of these agents, emphasizing the need to elucidate the molecular mechanisms of resistance.

Conclusion

Resistance to therapeutic monoclonal antibodies involves tumor-related and host-related factors. Determining clinically relevant resistance mechanisms is made difficult by the fact that several mechanisms of action are likely to be involved in the antitumor effect and that the antibodies are often used in combination with other agents. It is increasingly clear that resistance to mAbs will at least partly overlap resistance to conventional or novel small molecule anticancer agents. These findings underline the importance of understanding common resistance mechanisms and developing potent agents able to prevent the activation of survival pathways. Issues common to several, if not all, therapeutic mAbs involve the importance of the immunocompetent status of patients receiving therapeutic mAbs. While current immunomonitoring methods are not yet sufficiently standardized to adequately evaluate immunocompetence on a routine basis, it is likely that such a pretherapeutic evaluation will be useful in the future to define which patients are most at risk to benefit from or fail mAb therapy. Another important question concerns the potential impact of medications associated with therapeutic mAbs. For example steroids, potent immunosuppressive agents, are likely to reduced the role of accessory cells. It will be important to determine whether these or other associated agents influence the cytotoxic effect of mAbs in the clinic.

In spite of these limitations, our understanding of how and why therapeutic mAbs work or fail has made tremendous progress in the short period since these agents have become available. Further investigations will contribute to the development of more potent antibodies, sensitization strategies and optimal choice of therapeutic mAbs in individual patients in clinico.

Acknowledgements

Lina Reslan benefits from financial support from the Lebanese CNRS.

Conflicts of interest

C.D. received research funding from Roche.

References

1. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002; 346:235-42.
2. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Cazcman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16:2825-33.
3. Davis TA, Grillo-Lopez AJ, White CA, McLaughlin P, Cazcman MS, Link BK, et al. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment. J Clin Oncol 2000; 18:5135-43.
4. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976; 455:152-62.
5. Lobo ED, Hansen RJ, Balhbasar JP. Antibody pharmacokinetics and pharmacodynamics. Journal of pharmaceutical sciences 2004; 93:2645-68.
Understanding and circumventing resistance to anticancer monoclonal antibodies

6. Wood AM. Rituximab: an innovative therapy for non-Hodgkin’s lymphoma. Am J Health Syst Pharm 2001; 58:215-29.

7. Tedder TF, Engel P. CD20: a regulator of cell cycle progression of B lymphocytes. Immunol Today 1994; 15:450-4.

8. Jazirehi AR, Vega MI, Bonavida B. Development of rituximab-resistant lymphoma clones with altered cell signaling and cross-resistance to chemotherapy. Cancer Res 2007; 67:1270-81.

9. Jazirehi AR, Huerta-Yepez S, Cheng G, Bonavida B. Rituximab (chimeric anti-CD20 monoclonal antibody) inhibits the constitutive nuclear factor-(kappa)B signaling pathway in non-Hodgkin’s lymphoma B-cell lines: role in sensitization to chemotherapeutic drug-induced apoptosis. Cancer Res 2005; 65:264-76.

10. Cragg MS, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. Blood 2004; 103:2738-43.

11. Di Giardino N, Giretra N, Riva N, Vecchi A, Grigio V, Scanziani E, et al. Complement activation determines the therapeutic activity of rituximab in vivo. J Immunol 2003; 171:1581-7.

12. Golay J, Giretra N, Di Gaetano N, Mangaminis M, Mosca M, Nebuloni M, et al. The role of complement in the therapeutic activity of rituximab in a murine B lymphoma model homing in lymph nodes. Haematologica 2006; 91:176-83.

13. Hendra-Iliazilairu FJ, Japudy V, Outhorj J, Olajegha E, Huberman A, Repayku E, et al. Neutrophils contribute to the biological antitumor activity of rituximab in an in vivo model of non-Hodgkin’s lymphoma severe combined immunodeficiency mouse model. Clin Cancer Res 2003; 9:5866-73.

14. Czuczman MS, Olejniczak S, Gowda A, Kotowski A, Binder A, Kaur H, et al. Acquirement of rituximab resistance in lymphoma cell lines is associated with both posttranscriptional and posttranslational levels. Clin Cancer Res 2008; 14:6907-703.

15. Jais JP, Haioun C, Molina TJ, Rickman DS, de Reynies A, Berger F, et al. The expression of 16 genes related to the cell of origin and immune response predicts survival in elderly patients with diffuse large B-cell lymphoma treated with CHOP and rituximab. Haematologica 2007; 92:127-30.

16. Dayde D, Ternant D, Ohmesser M, Lerondel S, Pesnel S, Wafter H, et al. Tumor burden influences exposure and response to rituximab: pharmacokinetic—pharmacodynamic modelling using a syngeneic bioluminescent murine model expressing human CD20. Blood 2008; 113:5763-72.

17. Czuczman MS, Olejniczak S, Gorbaty S, Barnouse M, Peschel C. GM1 ganglioside expression sensitizes malignant B cells to apoptosis induced by rituximab (rituxan, anti-CD20 mAb) in non-Hodgkin’s lymphoma: implications in chemosensitization and therapeutic intervention. Oncogene 2005; 26:3629-36.

18. Glennie MJ, Hendra-Iliazilairu FJ, Clements J, Czuczman MS. Acquired resistance to rituximab is associated with chemotherapy resistance resulting from decreased Fas and Bax expression. Clin Cancer Res 2008; 14:1550-60.

19. Leneux L, Laurent C, Rigou M, Blance A, Olive D, et al. PKCzeta mTOR pathway: a new target for rituximab therapy in follicular lymphoma. Blood 2008; 111:285-91.

20. Vega MI, Huerta-Yepez S, Jazirehi AR, Garban H, Bonavida B. Rituximab (chimeric anti-CD20) sensitizes B-NHL cell lines to Fas-induced apoptosis. Oncogene 2005; 24:8114-27.

21. Suzuki E, Umezawa K, Bonavida B. Rituximab inhibits the constitutively activated PI3K-Akt pathway in B-NHL cell lines: involvement in chemosensitization to drug-induced apoptosis. Oncogene 2007; 26:6184-93.

22. Bonavida B. Rituximab-resistant induced antiapoptotic cell survival pathways: implication in chemoresistance. Blood 2007; 111:285-91.

23. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

24. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

25. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

26. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

27. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

28. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

29. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

30. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

31. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

32. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

33. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.

34. Czuczman MS, Olejniczak S, Ghorbani J, Amin S, Rigou M, Blance A, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43.
55. Valbohmmer D, Zhang W, Gordon M, Yang DY, Yan J, Press OA, et al. Molecular deter-
minants of cetuximab efficacy. J Clin Oncol 2005; 23:3536-44.

56. Wheeler DL, Huang S, Kruse TJ, Necerbekhi MM, Armstrong EA, Benavente S, et al. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. Oncogene 2008; 27:3944-56.

57. Benavente S, Huang S, Armstrong EA, Chi A, Hsu KT, Wheeler DL, et al. Establishment and characterization of a model of acquired resistance to epidermal growth factor recep-
tor targeting agents in human cancer cells. Clin Cancer Res 2009.

58. DiAugustine RP, Richards RG, Sebastian J. EGF-related peptides and their receptors in
lymphoid malignancies. Blood 2003; 101:3413-5.

59. Normanno N, Giardello F. EGF-related peptides in the pathophysiology of the
lymphoid gland. J Mammary Gland Biol Neoplasia 1997; 2:109-17.

60. Ménard S, Fortis S, Castiglioni F, Agresti R, Balsari A. HER2 as a Prognostic Factor in
Breast Cancer. Oncology 2001; 61:67-72.

61. Arlould G, Gelly M, Renault-Llorca F, Benoit L, Bonnetain F, Migeon C, et al. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? British journal of cancer 2006; 94:259-67.

62. Musolin O, Naldi L, Bortesi L, Pezzuolo D, Capelletti M, Missale G, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzu-
mab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 2008; 26:1789-96.

63. Price-Schiavi SA, Jepson S, Li P, Arango M, Rudland PS, Yee L, et al. Rat Muc4 (sia-
loomucin complex) reduces binding of anti-ErbB2 antibodies to tumor cell surfaces, a potential mechanism for herceptin resistance. International journal of cancer 2002; 99:78-93.

64. Nagy P, Friedlander E, Tanner M, Kapanen AI, Carraway KL, Isola J, et al. Expression of the NEU/HER-2 receptor is decreased on tumors from patients treated with trastuzumab. Cancer Res 2008; 68:597-604.

65. Vallbohmer D, Moessner E, Bruenker P, Unsin G, Puentener U, Suter T, et al. Novel 3rd gen-
eration humanized Type II CD20 antibody with glycoengineered Fc and modified elbow hinge for enhanced ADCC and superior apoptosis induction. Blood 2006; 108:229.

66. Dalle S, Reslan L, Brunet-Manquart S, Klein C, Umama P, Dumontier C. Compared antitumor activity of GA101 and trastuzumab against the human RL follicular lymphoma xenografts in SCID beige mice. Blood 2008; 1585:

67. Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell 2002; 2:127-37.

68. Konecny GE, Pegram MD, Venkatesan N, Finner R, Yang G, Rahmeh M, et al. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. Cancer Res 2006; 66:1630-9.

69. Nahra R, Yuan LX, Du Y, Esteve FJ. Lapatinib induces apoptosis in trastuzumab-
resistant breast cancer cells: effects on insulin-like growth factor 1 signaling. Molecular Cancer Ther 2007; 6:667-74.

70. Saadeh CE, Lee HS. Panitumumab: a fully human monoclonal antibody with activity in metastatic colorectal cancer. The Ann Pharmacother 2007; 41:606-13.

71. Meira DD, Nobrega I, de Almeida VH, Moroso JS, Cardoso AM, Silva RL, et al. Different antiproliferative effects of matumab and cetuximab in A431 cells are associ-
ated with persistent activity of the MAPK pathway. Eur J Cancer 2009; 14:119-24.

72. Saliz LB, Lenz HJ, Kindlir HL, Hochster HS, Wadler S, Hoff PM, et al. Randomized phase II trial of cetuximab, bevacinumab and irinotecan compared with cetuximab and bevacinumab alone in irinotecan-refractory colorectal cancer: the BOND-2 study. J Clin Oncol 2007; 25:4557-61.

73. Giarrino MJ, Schneider CJ, Hosford MA, Brahmer JR, Rudin CM, Finckenstein FG, et al. Dual Inhibition of the Epidermal Growth Factor Receptor Pathway with Cetuximab and Erlotinib: A Phase I Study in Patients with Advanced Solid Malignancies. Oncologist 2009; 14:119-24.

74. Matar P, Rojo F, Cassia R, Moreno-Bueno G, Di Cosimo S, Tabernerio J, et al. Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. Clin Cancer Res 2004; 10:6487-501.

75. Kim HP, Han SW, Kim SH, Im SA, Oh DY, Bang YJ, et al. Combined lapatinib and cetuximab enhance cytotoxicity against gefitinib-resistant lung cancer cells. Mol Cancer Thera 2008; 7:607-15.

76. Davis TA, Maloney DG, Grillo-Lopez AJ, White CA, Williams ME, Weiner GJ, et al. Combination immunotherapy of relapsed or refractory low-grade or follicular non-
Hodgkin’s lymphoma with rituximab and interferon-alpha-2a. Clin Cancer Res 2000; 6:6244-52.

77. Sacchi S, Federico M, Vitolio U, Boccomini C, Valiisa D, Baldini L, et al. Clinical activity and safety of combination immunotherapy with IFN alpha 2a and Rituximab in patients with relapsed low grade non-Hodgkin’s lymphoma. Haematologica 2001; 86:951-8.

78. Ansell SM, Witzig TE, Kier JN, Sloan JA, Jelinek DF, Howell KG, et al. Phase 1 study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin’s lymphoma. Blood 2002; 99:67-74.

79. Friedberg JW, Neuberg D, Gribbin JG, Fisher DC, Canning C, Koval M, et al. Combination immunotherapy with rituximab and interleukin 2 in patients with relapsed or refractory follicular non-Hodgkin’s lymphoma. British journal of haematology 2002; 117:828-34.

80. Umana P, Sacchi S, Federico M, Vitolio U, Boccomini C, Valiisa D, Baldini L, et al. Clinical activity and safety of combination immunotherapy with IFN alpha 2a and Rituximab in patients with relapsed low grade non-Hodgkin’s lymphoma. Haematologica 2001; 86:951-8.

81. Davis TA, Maloney DG, Grillo-Lopez AJ, White CA, Williams ME, Weiner GJ, et al. Combination immunotherapy of relapsed or refractory low-grade or follicular non-Hodgkin’s lymphoma with rituximab and interferon-alpha-2a. Clin Cancer Res 2000; 6:6244-52.

82. Ansell SM, Witzig TE, Kier JN, Sloan JA, Jelinek DF, Howell KG, et al. Phase 1 study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin’s lymphoma. Blood 2002; 99:67-74.

83. Friedberg JW, Neuberg D, Gribbin JG, Fisher DC, Canning C, Koval M, et al. Combination immunotherapy with rituximab and interleukin 2 in patients with relapsed or refractory follicular non-Hodgkin’s lymphoma. British journal of haematology 2002; 117:828-34.

84. Umana P, Sacchi S, Federico M, Vitolio U, Boccomini C, Valiisa D, Baldini L, et al. Clinical activity and safety of combination immunotherapy with IFN alpha 2a and Rituximab in patients with relapsed low grade non-Hodgkin’s lymphoma. Haematologica 2001; 86:951-8.

85. Ansell SM, Witzig TE, Kier JN, Sloan JA, Jelinek DF, Howell KG, et al. Phase 1 study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin’s lymphoma. Blood 2002; 99:67-74.