

Anti-cancer immune response mechanisms in neoadjuvant and targeted therapy

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Abstract Several studies suggest that the progression of malignant tumors as well as the response to chemotherapy and targeted therapy is critically dependent on the immunological parameters that are derived from the host immune system as well as a modulation of the immune system by therapeutic antibodies. It has been shown for many tumor types that the presence of a lymphocytic infiltrate in different types of cancers is a positive factor for clinical outcome and that the response to neoadjuvant chemotherapy is increased in a tumor with a prominent pretherapeutic infiltrate. Furthermore, new targeted therapies in breast cancer, such as trastuzumab, as well as in hematological malignancies, such as rituximab and alemtuzumab, have been shown to interact with immunological pathways, and this interaction is critical for response and clinical outcome. In neoplasms of lymphoid and hematopoietic tissues, targeted therapies not only reduce toxic effects on normal tissues but also lead to modulations of the immune system depending on the target molecule, its physiological function and cellular distribution. This review gives an overview on clinical data on response to classical chemotherapy as well

as molecular targeted therapy and its interaction with the immune system.

Keywords Neoadjuvant · Chemotherapy · Breast cancer · Lymphoma · Response · Lymphocytes

Introduction

Patients with breast cancer as well as malignancies of lymphoid and hematopoietic cells have benefited from the advances in cancer treatment over the past several decades. New approaches include the use of neoadjuvant chemotherapy as well as new molecular targeted drugs, many of which are therapeutic antibodies.

The inhibition of the transmembrane human epidermal growth factor receptor 2 (HER2) is an established treatment option not only in HER2-positive breast cancer [1] but also in other types of cancer, such as urothelial carcinoma [2] and gastric cancer [3]. The drug of first choice for HER2 targeting is trastuzumab (Herceptin[®], Genentech Inc./Hoffman-La Roche Ltd.), a recombinant humanized monoclonal antibody directed against the extracellular domain of HER2 [4]. Apart from trastuzumab, small molecule inhibitors like lapatinib (Tykerb[®], GlaxoSmithKline) or novel antibodies such as trastuzumab-DM1 (Genentech Inc.), an antibody-toxin conjugate, or the pan-HER inhibitor pertuzumab (Onmitarg[®], Genentech Inc.) are available but restricted to use in trastuzumab-refractory breast cancer or are tested in clinical studies to date. As most targeted tumor therapeutics, trastuzumab is used in conjunction with chemo- and radiotherapies and in this setting yields response rates from about 30% in breast cancer [5]. The clinical effect of trastuzumab is largely ascribed to HER2 pathway inhibition that results in the suppression of pro-

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proliferative and anti-apoptotic pathways. However, altered immune function influences the response to trastuzumab additionally to pathway inhibition. Thus, despite a trastuzumab resistance acquired *in vitro*, breast cancer cells remain sensitive to trastuzumab after transplantation into nude mice due to an activation of the immune response [6]. The major immunological mechanisms identified to be related to HER2 targeting by monoclonal antibodies are reviewed hereafter.

In hematological malignancies, despite increased remission rates, tumor cell resistance and life-threatening toxic effects on normal tissue limit the conventional chemotherapy. The toxic effects, especially, which limit the optimal dosing, are due to the lack of specificity for hematopoietic cells and particular lymphocyte populations [7]. Therefore the concept of targeted therapy is a promising approach for an effective therapy. Regarding hematopoietic and lymphoid malignancies, there are two different specific pathways: (i) the specific inhibition of small molecules like tyrosine kinase activity, e.g., in chronic myelogenous leukemia (CML) and (ii) the use of distinct cytotoxic monoclonal antibodies against cell surface molecules like anti-CD20 (rituximab) and anti-CD52 (Campath-1H).

Classical chemotherapy and therapy response

It has been shown for many tumor types that the presence of a lymphocytic infiltrate in different types of cancers is a positive factor for clinical outcome [8–12]. There is evidence from animal experiments that the immune system participates in the elimination of tumor cells and the control of tumor growth [13, 14]. Mechanisms of the tumor–immune interaction in response to chemotherapy have been investigated in several studies. It has been shown that anthracyclines are immunogenic and that calreticulin as well as the high-motility-group box 1 are mediators of this immunogenicity. Apetoh et al. have shown that a polymorphism of the toll-like receptor 4 (TLR 4) is an independent prognostic factor in response to chemotherapy [15]. TLR 4 is involved in an immunological response to microbial pathogens, as well as to endogenous stress signals that are released from dying tumor cells [16–18].

Taken together, these investigations suggest that the pretreatment host response may enhance the ability of chemotherapy to eliminate cancer cells. To investigate this hypothesis in the setting of clinical neoadjuvant multicenter trials in breast cancer, we have studied the lymphocytic infiltrate in tumor tissue of pretherapeutic core biopsies as a predictor of response to neoadjuvant chemotherapy.

Despite comparably high clinical response rates, only 10–25% of patients that are treated with neoadjuvant chemotherapy achieve a pathologically confirmed complete remission [19, 20]. Neoadjuvant chemotherapy constitutes an *in vivo* chemosensitivity test. Therefore, the pathological

complete remission (pCR) is a strong indicator of benefit from chemotherapy [21]. Pretherapeutic core biopsies provide an excellent basis for the analysis of predictive biological factors, to identify those patients who benefit most from chemotherapy.

We have studied pretherapeutic core biopsy samples from a total of 1,058 patients [22] enrolled in two large neoadjuvant studies, the GeparDuo and the GeparTrio, and investigated the presence of a lymphocytic infiltrate in the tumor stroma as well as inflammatory cells which are directly infiltrating the tumor cell nests.

In the GeparDuo study, a pCR was observed primarily in those tumors with an increased lymphocytic infiltrate. The overall pCR rate was 12.8% while the pCR rate for the cases with increased intratumoral lymphocytes (>10%) was 31% ($p < 0.0005$, two-sided Fisher test). The subgroup of lymphocyte-predominant breast cancer (LPBC), defined as those cases with more than 60% either stromal or intratumoral lymphocytes, had a pCR rate of 41.7% ($p < 0.0005$, chi-square test). Tumors with a focal infiltrate had a pCR rate of 10.8%, while those tumors without any infiltrating lymphocytes had a pCR rate of only 2.8%. Figure 1 a–d shows a pCR case with a prominent lymphocytic infiltrate, while Fig. 1 c and f shows a typical carcinoma without such an infiltrate; this case did not have a pCR.

The results were further validated using 840 cases from the GeparTrio trial. Here the lymphocytic infiltrate was a strong predictor of pCR in univariate ($p < 0.0005$) and multivariate logistic regressions ($p = 0.001$).

Furthermore, molecular markers of lymphocyte recruitment and infiltration in breast cancer tissue were evaluated by kinetic PCR using RNA isolated from the formalin-fixed paraffin-embedded (FFPE) core biopsies (Fig. 2). These results are the basis for predictive signatures that use immune-related markers to predict the response to neoadjuvant chemotherapy.

Taken together, the concept of a contribution of the immune response to the success of chemotherapy is strongly supported by our analysis. According to this concept, the destruction of tumor cells by chemotherapeutic agents may release tumor-associated antigens. This could trigger an immune response directed against the tumor cells which will be particularly strong in those cases where a sensitization of the immune system against some tumor antigens is present before the onset of chemotherapy. Therefore, chemotherapy might act as a functional immunotherapy in those tumor types, and the combination of chemotherapeutic destruction of tumor cells as well as increased immune response could lead to a pathological complete remission [23].

Our study identifies a subgroup of breast carcinomas with a prominent lymphocytic infiltrate in the tumor tissue and a particularly strong response to chemotherapy. We

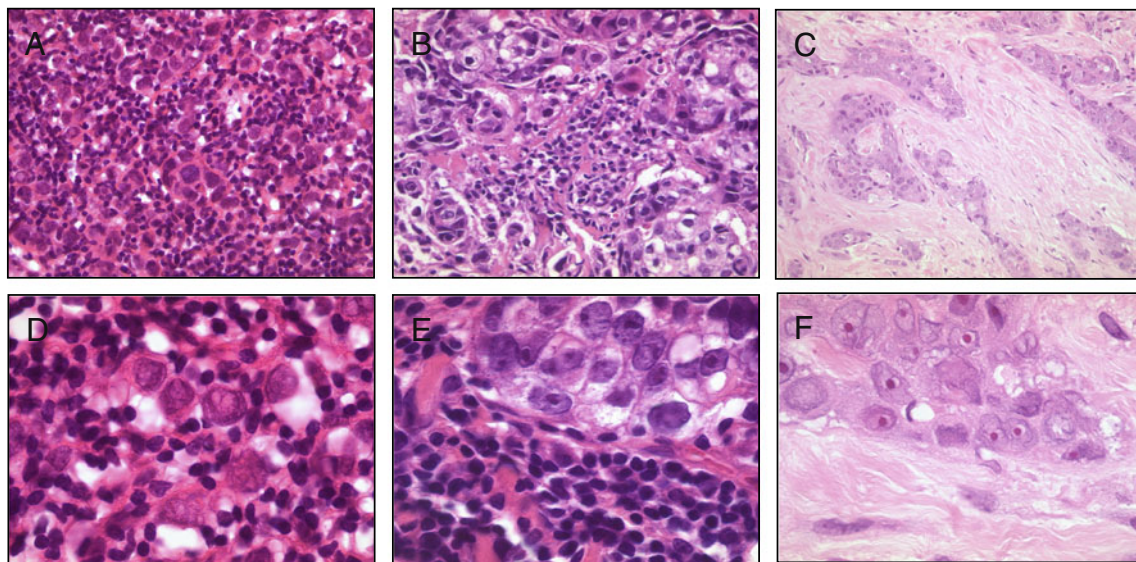


Fig. 1 Infiltration of lymphocytes in breast cancer tissue. Increased intratumoral (iTU-Ly) (a, d) as well as stromal (str-Ly) (b, e) lymphocytic infiltrate in core biopsies from tumors with a pCR after chemotherapy. For comparison, a typical histology of a breast cancer

without a lymphocyte infiltrate is shown (c, f); this case did not show a pCR (modified with permission from [22] © 2008 American Society of Clinical Oncology. All rights reserved)

have used the term “lymphocyte-predominant breast cancer (LPBC)” for those tumors with a particularly strong lymphocytic infiltrate; those tumors have an increased response to neoadjuvant chemotherapy. However, even tumors with a less prominent but detectable lymphocytic infiltrate show an increased response; therefore, we suggest that the response to chemotherapy is dependent on the lymphocytic infiltrate as a continuous parameter, as seen in the logistic regression as well as in comparison of subgroups with different percentages of lymphocytes. LPBC should thus be used as a working category to

indicate an increased odds ratio for pathological complete response rather than a separate tumor entity.

While our primary analysis was performed solely by morphological analysis of standard H&E sections, we used the isolation of RNA from FFPE tissue as well as RT-PCR to show that the gene expression of chemoattractant mediators for B and T cells is present in the tumor tissue and that in particular the T-cell markers are linked to the chemotherapy response.

Gianni et al. have studied RNA markers in 89 breast carcinomas treated with neoadjuvant chemotherapy, of

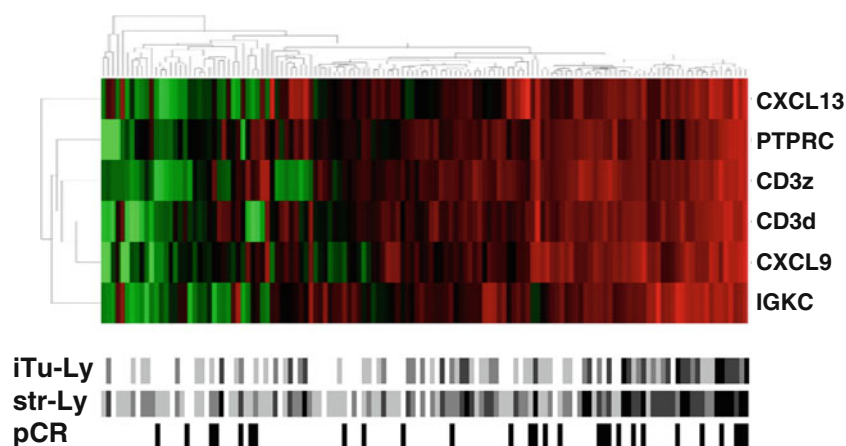


Fig. 2 Evaluation of gene expression of immune biomarkers in 134 formalin-fixed paraffin-embedded core biopsies from breast cancer cases treated with neoadjuvant chemotherapy. RNA was isolated from FFPE tissue, and RT-PCR was performed. Cluster analysis shows a co-regulation of several inflammatory genes related to a B-cell or

T-cell infiltrate. The results indicate that a molecular pattern can be detected in tumor tissue that reflects the activity of the immunological response and that might be used as a basis for development of further predictive signatures (modified with permission from [22] © 2008 American Society of Clinical Oncology. All rights reserved)

which 11 had a pCR and have shown that immune-related genes, such as CD3, are linked to the response to chemotherapy [24]. Very recently, an increased expression of CD3 has been described in ten patients with a complete response to neoadjuvant chemotherapy in a cohort of 73 patients, which is in line with our results [25]. Several studies have suggested the possible mechanisms of the tumor–immune interaction in response to chemotherapy. A reduction of Foxp3 T cells was observed in 56 tumors treated with neoadjuvant chemotherapy [26] and has been linked to a poor prognosis and therapy response in breast cancer [27–29]. Tregs are a subset of CD4+ T-helper cells detectable by the expression of CD25, CTLA-4, GITR, and Foxp3. They display anergy when stimulated by T-cell receptor cross-linking and inhibit cytotoxic T cells, T-helper cells, dendritic cells (DC), NK cells, and NK T and B cells [30]. Increased levels of circulating and intratumoral Treg levels are found in patients harboring different tumors, such as lung, ovarian, [31] breast, and pancreatic cancers [32]. The presence of Foxp3 regulatory T cells (Tregs) has been linked to a poor prognosis in breast cancer [27–29]. In addition, Macmillan and his group have shown that systemic markers of inflammation also have prognostic value in different types of cancer [33–37].

There are two possible conclusions from this concept for the management of breast cancer patients. In the diagnostic setting, the immunological parameters might be integrated in predictive signatures for the response to neoadjuvant chemotherapy. This is particularly interesting because the immunological parameters are independent of the classical predictive markers, such as grading or hormone receptor status. Several prognostic and predictive signatures in breast cancer already contain biomarkers of tumor-associated inflammation and immune response. A prospective evaluation of the lymphocytic infiltrate as a predictor of the response to neoadjuvant chemotherapy is currently performed in the Predict substudy of the GeparQuinto trial of the German Breast Group.

As an additional conclusion, in the therapeutic setting, there is the notion that chemotherapeutic approaches are not only immunosuppressive but that a partial activation of the immune system is an important element of an efficient activity of the chemotherapy against the tumor cells. As a consequence, therapeutic concepts that combine chemotherapy with an additional immunomodulatory activity should be considered and tested.

A similar concept that has already shown clinical efficacy is the combination of therapeutic antibodies and classical chemotherapy. From those approaches we have learned a lot about the contribution of immune mechanisms to chemotherapy response. In the following parts of this review, we will discuss examples of targeted therapy in breast cancer as well as lymphoma.

Immunological mechanisms of trastuzumab treatment of HER2-positive tumors

Innate immune responses—ADCC Among the immunological mechanisms related to trastuzumab treatment of HER2-positive disease, antibody-dependent cell-mediated cytotoxicity (ADCC) is the best characterized one. ADCC is a strategy of the innate immune system when antibody-coated cells are lysed by effector cells that bind IgG via IgG Fc receptors (FcγR). FcγR are present on monocytes/macrophages, natural killer (NK) cells, and granulocytes [38]. Human FcγR are composed of three distinct classes, FcγRI, FcγRII, and FcγRIII. FcγRIIIa is expressed on NK cells, and FcγRIIa is expressed on dendritic cells [39]; however, little is known about the role of inhibitory FcγRIIb in cancer. The binding of trastuzumab to HER2 on tumor cells attracts the above-mentioned immune effector cells to the tumor cell and leads to a rapid cytolysis. The evidence that ADCC is one major mechanism of an immune response to trastuzumab in animal models and in vitro came from very early studies [40–42] and was stressed by the finding that mice deficient in ADCC and in FcγR responded weakly to trastuzumab [43]. As ADCC is mediated by multiple effector cells and there is considerable genetic variability of FcγR, the individual capacity of reacting to trastuzumab with ADCC is different. Thus, special variants of FcγR, such as FcγRIIIa-158 V/V and FcγRIIa-131 H/H or the combination of both, have been shown to elicit an increased ADCC and were associated with a clinical response to trastuzumab and survival [44], and the FcγR variant CD16 V/F was related to an increased killing capacity of NK cells [45]. The conjugate trastuzumab–DM1 was also found to elicit ADCC [46], whereas no reports on the immune functions of the other HER2-inhibiting substances are available to date. Further evidence of an impact of ADCC/NK cell function on the clinical outcome was provided by the finding that clinical responders to trastuzumab had higher levels of ADCC and NK cell activity than non-responders and that survival was dependent on sustained NK cell function [47]; however, the definitive proof of a clinical relevance of ADCC and the time frame during which it may be effective (only an early response or a long-term effect also?) need further study.

Adaptive immune responses: HER2-specific T-cell activation There is increasing evidence that the adaptive immune system plays a major role in the response and resistance to trastuzumab. The binding of trastuzumab to HER2 on the cell surface triggers the internalization and degradation of the tumor antigen and subsequently the processing and antigen presentation which can be performed by tumor cells or by antigen-presenting cells, such as dendritic cells (CD

after transportation of HER2 fragments to draining lymph nodes following apoptosis of tumor cells and release of the tumor antigen [48, 49]. Antigen presentation to matched specific lymphocytes on major histocompatibility complex class I (MHC class I) molecules elicits a specific adaptive immune response resulting in the maturation of cytotoxic CD8+ T cells and CD4+ T-helper cells. Taylor et al. showed that breast cancer patients treated with trastuzumab develop a CD4+ T-helper cell response [50], and HER2-specific cytotoxic T cells in addition to trastuzumab were able to kill HER2-positive esophageal carcinoma cells [51]. In a murine mammary tumor model, it has been shown that antibody binding to HER2 enhances CD8+ T-cell function against the tumor [52]. In adaptive immunity, FcγR play a major role too, as the antigen-presenting function of DC is dependent on their activating FcγR I-III. Although not investigated so far, it is likely that FcγR polymorphisms are also relevant in the specific immune response to HER2 overexpressing tumors treated with trastuzumab, which might constitute an additional factor contributing to the interindividually varying effectivity of trastuzumab. Interestingly, a subset of NK cells, characterized by high CD56 expression, is involved not only in innate but also in adaptive immunity, as subsequently to trastuzumab treatment of breast cancer cells, it produces various T-helper type 1 cytokines that enhance antigen presentation by DC and chemotaxis of T cells [53]. DC in turn secrete cytokines that activate NK cells [54]. The cross-talk between DC and NK cells thus links the innate to the adaptive immune responses at the same time.

Effective antigen presentation is dependent on intact antigen presentation machinery (APM). Defects in the APM are frequently found in malignant tumors, and this constitutes one mechanism of tumor immunoescape. In esophageal adenocarcinoma cells, a deficiency of the transporter associated with antigen processing 2 was shown to impair cytotoxic T-cell response after trastuzumab that could be restored by treatment with IFN-γ [51]. Tumors even suppress DC function, the major players in antigen presentation. This has been shown by Whiteside et al., who found that components of the APM were downregulated in immature DC after incubation with tumor cells which was accompanied by a decreased antigen presentation and increased apoptosis of T cells [55].

These striking findings suggest that adaptive immune responses might be essential in the response and resistance to trastuzumab treatment of HER2-positive cancer and might, in particular, explain the long-term response to treatment. However, links to clinical parameters of response are lacking to date, and translational research projects on this area are necessary.

Role of regulatory T cells Within the group of breast cancer patients, circulating Foxp3-positive Tregs are particularly

elevated in patients with HER2-positive tumors, and trastuzumab treatment is able to reduce Treg/CD4+ [28] and Treg/Th17 ratio [56], respectively, which is associated with the clinical response in one of both studies [28]. The mechanisms behind Treg reduction by trastuzumab remains unclear to date; however, these studies demonstrate that apart from activating directly both innate and adaptive immune responses, trastuzumab treatment of HER2-positive cancer indirectly boosts immunosurveillance in tumor patients by reducing immunosuppressive processes.

Conclusion Treatment of HER2-overexpressing cancer with the monoclonal antibody trastuzumab activates innate and adaptive immune mechanisms both directly and indirectly. Evidence from in vitro and animal models argues for an essential role of the immune system in the response and resistance to trastuzumab treatment, additionally to the effect of HER2 pathway inhibition. Although some studies already linked these findings to clinical measures of outcome, further study is needed to definitely prove the clinical relevance of immunological functions of trastuzumab. Particularly, it would be of intriguing clinical utility to, on the one hand, use immunological parameters as predictive tools to therapy response, and, on the other hand, add immunological therapy, e.g., cytokines, to increase the effect of trastuzumab. Furthermore, as discussed in the section before, immunological processes are very likely to influence the response to conventional chemotherapy too. As trastuzumab is usually administered together with chemotherapy, immune parameters will be influenced by both therapeutic components. To what extent and how this happens has not yet been investigated and opens a fascinating field of research for the future.

Small molecules as inhibitors of tyrosine kinase activity in CML therapy

According to the WHO classification, CML is defined by the presence of a chromosomal translocation known as Philadelphia chromosome t(9;22) [57]. The result is a fusion of the *c-abl* oncogene from chromosome 9 and the breakpoint cluster region (*bcr*) from chromosome 22 which forms the *bcr-abl* gene [58]. The gene encodes a fusion protein with a constitutive tyrosine kinase activity, which leads to the activation of intracellular pathways especially for proliferation, survival, and adhesion of tumor cells [59]. Looking for signal transduction inhibitors, a specific platelet-derived growth factor receptor inhibitor (so called small molecule) was developed [7]. The signal transduction inhibitor 571 (CGP57 148B) later named imatinib mesylate (Glivec/GleevecTM) was found to be a selective inhibitor of

abl tyrosine kinase. This small cell molecule blocks ATP binding to the *bcr-abl* tyrosine kinase. The inhibition of tyrosine kinase activation results in an absence of phosphorylation of the substrates and a pathway disruption of the downstream events [60, 61]. Gleevec is applied as a useful therapeutic agent for patients with a diagnosis of CML in the chronic phase.

As an imatinib resistance or intolerance could be observed, a new generation of TKIs, like dasatinib (Sprycel), was developed. Dasatinib inhibits a broader spectrum of tyrosine kinases including EPH, TEC, and SRC which have physiological functions in immune responses [62].

It is well known that at diagnosis of CML, a lower proportion of B cells and dendritic cells and a higher proportion of NK cells are detected in bone marrow biopsies compared to healthy controls. While during imatinib (first generation tyrosine kinase inhibitor, TKI) therapy, these changes are normalized and lead to a similar immunoprofile compared to healthy controls, therapy with dasatinib (second generation TKI) showed a distinct and relevant immunoreaction [63]. Interestingly, a marked accumulation of lymphocytes in the bone marrow of patients with CML before treatment seems to be associated with a better prognosis.

Whereas in vitro data have shown that imatinib and dasatinib lead to the reversible inhibition of T-cell and B-cell activation, more recent studies could demonstrate that in vivo dasatinib more than imatinib induces immunostimulation in the form of the marked expansion of T and NK cells in a proportion of patients [64, 65]. This seems to be related with a good therapy response as well as with autoimmune-like side effects.

In particular, Rohon et al. [63] have recently analyzed the changes of the immunoprofile in the bone marrow (BM) and peripheral blood (PB) of CML patients at diagnosis and during TKI treatment. At the time of diagnosis, most leukocytes in the BM and PB are of granulocytic origin. The absolute number of monocytes and lymphocytes in PB is increased, reflecting increased cell proliferation in BM, characteristic of CML. During therapy with dasatinib, a significant proportion of patients had an increased lymphocyte and monocyte count, whereas patients with imatinib therapy showed lower counts. The distribution of CD4 and CD8 cells did not differ from healthy patients at the time of diagnosis, but after dasatinib, the ratio was lower compared to imatinib-treated patients. NK cells were elevated in BM and in PB at the time of diagnosis as well as during dasatinib therapy, but T cells were decreased. Furthermore, at the time of diagnosis and after imatinib therapy, the Tregs in PB and BM had a similar proportion as in the healthy controls, whereas these cells had a lower proportion in PB and BM after dasatinib treatment. Interestingly, dendritic cells were significantly decreased whereas lymphocyte

activation markers showed a similar distribution compared to the healthy controls at the time of diagnosis. Only CD57 expression was decreased, and CD62L antigen expression was lower in CD8+T cells than in the healthy controls. After dasatinib therapy, the patients could be divided into two groups. One who resembled the healthy controls and imatinib-treated patients and the other group with an immunoactivated phenotype that was characterized by elevated CD8+ lymphocytes, NK- and NKT-like cell counts, and increased expression of CD57, HLA-DR, Ki-67, and CD45RO antigens together with lower levels of CD62L antigen. Treg levels were decreased in this patient group which may contribute to an enhanced immune hyperreactivity to recurrent antigen stimulus. Careful follow-up is recommended in those patients with a large population of highly differentiated effector memory T cells as the reservoir of naïve lymphocytes is then smaller, and T-cell diversity declines. This could lead to an impaired ability to respond to heterologous infections or predispose patients to autoimmune-like disorders. Interestingly, immunosuppression and atypical infections like EBV-associated mucosal leukoplakia or *Pneumocystis carinii* pneumonia in CML patients are described in some patients treated with dasatinib [66].

In conclusion, TKIs of the different generations have significant differential effects on the immune effector cells. As tyrosine kinases are key regulators of immune responses, concerns on immunosuppressive effects of TKIs have been raised. The published data do not support an immunosuppressive effect in vivo. Whereas imatinib has obviously an immune neutral effect, dasatinib is more reminiscent of an immune response to a chronic, persistent antigen challenge in a proportion of patients [63].

Rituximab as a monoclonal antibody in targeted therapy Rituximab (RTX) is a genetically engineered chimeric monoclonal antibody directed against the CD20 membrane-associated glycoprotein. It is composed of a human IgG kappa constant region and a murine variable region recognizing CD20 [67]. This molecule is expressed in normal B cells as well as in most malignant neoplasms derived from B cells. A significant clinical benefit is observed not only in follicular lymphoma and diffuse large B-cell lymphoma but also in other settings like autoimmune cytopenias and rheumatoid arthritis. Early pro-B cells and most of the plasma cells do not express CD20; thus, lymphomas from these cells do not benefit from RTX therapy [68].

RTX takes effect by antibody-dependent cellular cytotoxicity, complement-mediated cell lysis, and cell growth inhibition or apoptosis. RTX is thought to inhibit B-cell survival and proliferation through negative regulation of

canonical signaling pathway involving AKT and ERK. Pretreatment with RTX results in a time-dependent inhibition of the B-cell receptor (BCR) signaling cascade involving Lyn, Syk, PLC gamma 2, Akt, ERK and calcium mobilization [69].

Moreover, recent studies could demonstrate that RTX interferes with BCR signaling by targeting proximal components of the BCR cascade, including decreased BCR expression and disturbances of BCR membrane dynamics.

Effective treatment with RTX results in a complete depletion of B cells from peripheral blood. B-cell regeneration starts between 6 and 10 months after depletion with newly performed B cells [67]. During this regeneration phase, a special B-cell subset could be observed: the transitional B cells. Transitional cells represent a crucial step in the differentiation and selection of the mature B-cell compartment. Their identification is based on the high level of expression of CD10, CD24, and CD38. Circulating transitional B cells were initially analyzed based on surface phenotype: IgD+CD27-CD38hiCD24hi and divided in T1, T2, and T3 cells. In normal peripheral blood, transitional B cells are detected at low levels. Late transitional B cells (T2 and T3) are found at comparatively increased frequencies in cord blood and spleen, but are relatively rare in bone marrow [70]. Notably, even more than a year after RTX-induced B-cell depletion, nearly all of the B cells in peripheral blood display a transitional phenotype.

Despite the success of rituximab (type I of anti-CD20 mAb), a substantial proportion of B-cell lymphomas is unresponsive or develops a resistance. A possible explanation is the CD20 modulation as a central role in determining the efficacy. It could be demonstrated that rituximab evokes a reduction of CD20 up to 90% whereas tositumomab-like anti CD20 (type II) left CD20 largely unchanged. Moreover, rituximab internalized with CD20 as it modulated from the cell surface which not only is limiting the therapeutic effect but complicates additionally the identification of residual lymphoma cells. After the RTX-containing therapeutic regimen, the tumor cells as well as the reactive B cells do not show any or only a partial expression of CD20. In these instances, the tumor cells have to be detected by other B-cell specific/characteristic antibodies like PAX5, CD79a, or CD19 (Fig. 3).

Alemtuzumab (Campath-1H) The humanized anti-CD52 antibody of the IgG1 subtype alemtuzumab belongs to the Campath-1 family of antibodies that target the human CD52 antigen, a 12-amino acid glycosylphosphatidylinositol-anchored cell surface glycoprotein [71, 72]. CD52 is expressed at high levels on both normal and neoplastic B and T lymphocytes [71, 73–76] and is also found at lower levels on monocytes/macrophages and eosinophils in addi-

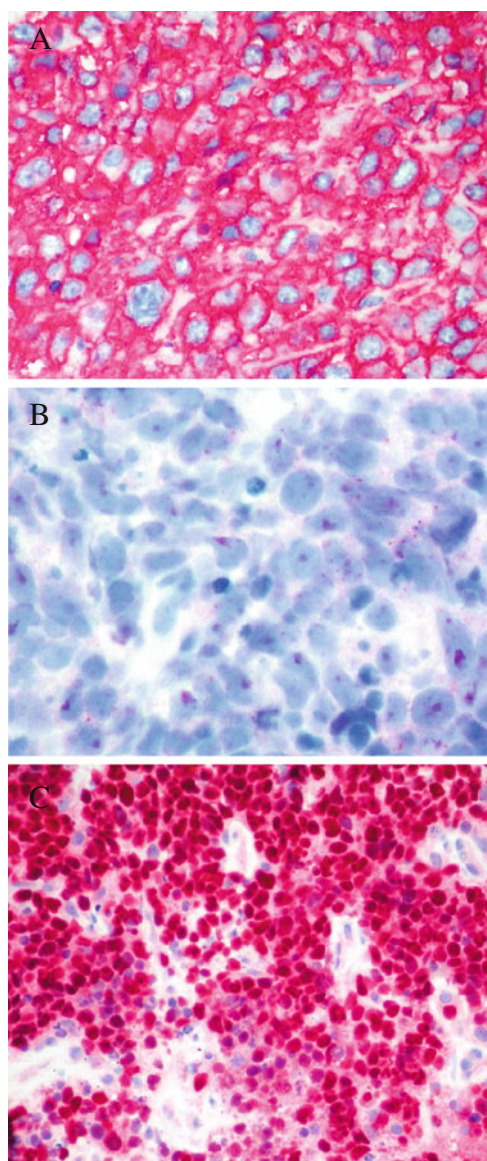


Fig. 3 **a** B-cell lymphoma with a membranous expression of CD20 on the neoplastic cells (immunostain using a monoclonal CD20 antibody). **b** Complete loss of CD20 expression after treatment with rituximab. **c** The B-cell origin of the neoplastic cells can still be detected by demonstration of pax-5 expression, a B-cell characteristic transcription factor showing nuclear labeling of the tumor cells

tion to the male reproductive tract (epithelia of the epididymis and duct deferens). The exact function of CD52 remains unclear, but some evidence suggests that it may be involved in T-cell migration and costimulation [77]. Radioisotopic studies have shown that up to 5% of lymphocyte cell surfaces are covered by CD52 molecules. It is of importance that CD52 is not expressed on CD34+ hematopoietic progenitor cells [75]; therefore, alemtuzumab does not interfere with early hematopoietic progenitor cell development. Little CD52 expression is present on mature natural killer cells and neutrophils.

The Campath-1 family of monoclonal antibodies was initially developed as T-cell depleting agents, and earlier studies have demonstrated considerable efficacy of these antibodies in preventing both GvHD and graft rejection in patients undergoing allogeneic stem cell transplantation. Alemtuzumab is indicated for the treatment of chronic lymphocytic leukemia (CLL) and has shown considerable activity in relapsed/refractory disease and also in previously untreated CLL. Alemtuzumab has also been found to have significant activity in T-cell neoplasms such as in mycosis fungoides as well as in T-prolymphocytic leukemia and is undergoing phase-III clinical trials for the treatment of multiple sclerosis [78, 79].

In vitro studies have indicated that both ADCC and complement-dependent cytotoxicity are the primary mechanisms of action for the Campath-1 family of antibodies [80]; also, caspase-independent apoptosis has been demonstrated in B-cell lymphoma cell lines and primary CLL cells treated with alemtuzumab [81]. The exact mechanism of action of alemtuzumab in vivo has been hampered however by the lack of cross-reactivity between human and mouse CD52. To address this issue, a transgenic mice expressing human CD52 was created [82]. It was shown that these mice did not display any phenotypic abnormalities and were able to mount normal immune responses. The tissue distribution of hCD52 and the level of expression by various immune cell populations were comparable to those seen in humans. Treatment with alemtuzumab replicated the depletion of peripheral blood lymphocytes observed in humans. Lymphocyte depletion was not as profound in lymphoid organs (spleen and lymph nodes), providing a possible explanation for the relatively low incidence of infection in alemtuzumab-treated patients. It has been hypothesized that preservation of the innate immune responses (NK cells, neutrophils) provides a relative defense against pathogens and that residual lymphocytes in lymphoid organs may also still be available to participate in the immune response. The pattern of lymphocyte repopulation in transgenic mice treated with alemtuzumab also mirrored that observed in humans: B lymphocyte recovery was most rapid with T lymphocytes repopulating more slowly and remaining at below normal levels for a prolonged period of time. Alemtuzumab does not deplete CD52-negative hematological precursors, which presumably accounts for the relatively rapid recovery of B lymphocytes. The partial loss of thymocytes observed in transgenic mice may contribute to the delay in T-cell recovery. As bone marrow stem cells are not affected by alemtuzumab, the long duration of T-cell lymphopenia has yet to be elucidated.

The potent T-cell depleting activity of alemtuzumab probably plays a role in the efficacy of this antibody in controlling disease in relapsing/remitting multiple sclerosis.

Additional factors may also play a role as the therapeutic benefit persists irrespective of T-lymphocyte repopulation. It has been therefore speculated that alemtuzumab treatment may create a “tolerogenic environment.” This is supported by the observation of a predominance of peripheral blood T cells with a regulatory phenotype during the first 6 months following alemtuzumab treatment in MS patients. This “sparing” of T cells with regulatory immunophenotype has also been observed in CD52 transgenic mice.

Interestingly, in vivo data in transgenic mice revealed that alemtuzumab effector mechanisms were (in contrast to in vitro data) largely independent of complement and appeared to be mediated by neutrophils and natural killer cells because removal of these populations strongly inhibited the activity of alemtuzumab, whereas removal of complement had no impact [82]. Neutrophil-mediated killing has been also reported as an effector mechanism of rituximab and may represent a more general mechanism of ADCC.

Taken together, interactions between immune cells and tumor cells are relevant for the response to therapy in various settings, from classical anthracycline-based chemotherapy to antibody-mediated therapy in breast cancer and other types of tumors. The synergistic effect between therapy and immune response might lead to interesting new study concepts as well as new diagnostic signatures that include parameters of the immune response.

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