Immunological Approaches in the Treatment of Metastasized Breast Cancer

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Key Words

Breast cancer · Metastasis · Antibody · Therapy

Summary

A better understanding of tumor biology has led to the development of a number of antibody-based targeted therapies in breast cancer. Several of these newer agents, such as trastuzumab and bevacizumab have demonstrated clinical activity and have improved the treatment of patients with metastatic breast cancer (MBC). Trastuzumab is a monoclonal antibody that binds to the extracellular domain of the HER2 receptor. The addition of trastuzumab to chemotherapy and also to endocrine therapy has enhanced efficacy of treatment. New antibody-based strategies directed against HER2 are under development. These new approaches include pertuzumab, an antibody with a different binding epitope that inhibits dimerization of HER2 with other members of the HER receptor family and TDM1, a trastuzumab-based antibody chemotherapeutic conjugate. Another approach to the treatment of solid tumors is inhibition of angiogenesis. The anti-VEGF antibody bevacizumab has been approved for treatment of MBC. Although the mechanism of action is still under investigation, bevacizumab is tested in other clinical settings such as adjuvant therapy, maintenance therapy, and in combination with both chemotherapy and other targeted agents. In this review, we will summarize the most important studies on trastuzumab and bevacizumab, and describe new antibodies currently under clinical development.

Schlüsselwörter

Mammakarzinom · Metastasen · Antikörper · Therapie

Zusammenfassung

Ein verbessertes Verständnis der Tumorbilogie hat zu der Entwicklung einer Vielzahl Antikörper-basierter zielgerichteter Therapieansätze beim Mammakarzinom geführt. Mehrere dieser neuen Ansätze wie Trastuzumab und Bevacizumab besitzen klinische Wirksamkeit und haben die Behandlungsmöglichkeiten des metastasierten Mammakarzinoms verbessert. Trastuzumab ist ein monoklonaler Antikörper, der an die extrazelluläre Domäne von HER2 bindet. Die Anwendung mit Chemotherapie und auch endokriner Therapie hat zu einer Wirkungsverstärkung dieser Ansätze geführt. Neue Antikörper-basierte Therapieansätze gegen HER2 sind gegenwärtig in der klinischen Prüfung. Beispiele hierfür sind die Antikörper Pertuzumab, ein Antikörper mit einer anderen Bindungsdomäne als Trastuzumab, der die Dimerisierung von HER2 inhibiert sowie TDM1, ein Trastuzumab-basierter Antikörper, der mit einem Chemotherapeutikum konjugiert ist. Ein weiterer Ansatz in der Therapie soliden Tumors ist die Inhibition der Angiogenese. Der Anti-VEGF-Antikörper Bevacizumab ist für die Behandlung des metastasierten Mammakarzinoms zugelassen. Obwohl der Wirkungsmechanismus noch Gegenstand von Forschungsprojekten ist, wird die Substanz in verschiedenen klinischen Situationen wie der adjuvanten Therapie, der Erhaltungstherapie und in Kombination mit anderen zielgerichteten Therapieansätzen erprobt. In dieser Übersichtsarbeit sollen die wichtigsten Studien mit Trastuzumab und Bevacizumab sowie mit neuen Antikörpern erwähnt werden, die sich gegenwärtig in der klinischen Testung befinden.

Metastatic Breast Cancer: Need for New Therapeutic Options

In metastatic breast cancer (MBC), no definite cure seems possible with current treatment options, although long-term survival is observed. This situation has not changed over the past decades, despite progress in the field of chemotherapy and endocrine treatment. Therefore, there is a strong need for new approaches; one of these is the therapeutic use of antibodies. This article summarizes antibody-based therapy already established in the treatment of MBC, and briefly describes new agents currently in clinical and preclinical testing.

HER2 as Biologic Factor in Breast Cancer Biology and Rationale for Targeting HER2 with Therapeutic Approaches

The discovery and characterization of the human epidermal growth factor receptor 2 (HER2) in 1979 led the way towards a breakthrough in the treatment of breast cancer. HER2 is a growth factor receptor with a molecular weight of 185 kD and extra- and intracellular domains. HER2 is overexpressed in 15–20% of primary breast cancers. Breast tumors that exhibit HER2 protein overexpression or gene amplification are more aggressive and more likely to recur. It was demonstrated that HER2 plays an important role in regulating cell proliferation, angiogenesis, invasion, and metastatic properties of tumor cells [1–3]. Gene expression profiling analyses indicate that HER2-positive breast cancer seems to be a distinct disease entity [4], with a characteristic pattern of gene expression that differs from the luminal and triple negative subtypes. Prior to the development of HER2 targeted therapy, HER2-positive breast cancer was associated with an aggressive clinical course characterized by resistance to traditional systemic therapy [5]. Therefore, the idea of targeting HER is obvious. In this article, we focus on antibody-based approaches for the targeting of HER2.

Trastuzumab as Antibody-Based Approach for Targeting HER2

Several monoclonal antibodies have been approved for the treatment of various cancer entities. One of the pioneering antibodies was trastuzumab (Herceptin®, Roche Pharma AG, Grenzach-Whylen, Germany), the first treatment targeting HER2 approved for the treatment of HER2-positive MBC. Trastuzumab was developed from murine monoclonal antibodies against HER2 [6, 7]. A clone called 4D5 showed inhibitory activity in HER2-overexpressing breast cancer models. The humanized variant of 4D5 is now known as trastuzumab. Trastuzumab binds to the extracellular region of the HER2 receptor. The mechanisms of action of trastuzumab are exerted both via the extracellular and intracellular domains of the protein and involve antibody-dependent cell-mediated cytotoxicity and also HER2 receptor inhibition [8–11]. Actions via the intracellular domain lead to downstream signal transduction inhibition, reduction of angiogenesis, and impaired cleavage of the extracellular receptor domain [9, 12, 13]. Results from experimental studies suggest additional mechanisms of action like inhibition of chemotherapy-induced or radiation-induced DNA damage repair [14–16].

Clinical Studies with Trastuzumab in Metastatic Breast Cancer

In phase II trials, trastuzumab monotherapy was effective and well tolerated as both first-line therapy and also after chemotherapy [17, 18] (table 1). In a phase III trial of first-line trastuzumab in combination with chemotherapy (anthracycline/cyclophosphamide or paclitaxel), a significant improvement in response rate, time to disease progression, and overall survival was observed when compared with chemotherapy alone [19] (table 1). These phase II and III results led to approval of trastuzumab as monotherapy in patients who previously received

Table 1. Examples of studies with trastuzumab in metastatic breast cancer

Trial design	Phase	ORR, %	TTP, median, mo	OS, median, mo
Trastuzumab monotherapy, first-line [17]	II	26	3.5	22.9
Trastuzumab monotherapy after chemotherapy [18]	II	21	3.1	13.0
Trastuzumab plus letrozole as first- or second-line therapy [29]	II	26	5.8	nr
Paclitaxel, carboplatin plus trastuzumab (every 3 weeks) [25]	II	65	9.9	27.6
Paclitaxel, carboplatin plus weekly trastuzumab [25]	II	81	13.8	38.4
Trastuzumab, docetaxel, and cisplatin [26]	II	79	9.9	nr
Trastuzumab, docetaxel, and carboplatin [26]	II	58	12.7	nr
Trastuzumab plus docetaxel [20] vs. docetaxel alone	II	61/34	11.7/6.1	31.2/22.7
Trastuzumab plus chemotherapy vs. chemotherapy alone [19]	III	50/32	7.4/4.6	22.1/18.4
Trastuzumab, paclitaxel plus carboplatin vs. trastuzumab plus paclitaxel [27]	III	52/36	nr	35.7/32.2
Anastrozole with trastuzumab vs. anastrozole alone [30]	III	20.3/6.8	4.8/2.4	28.5/23.9

ORR = Overall response rate; TTP = time to progression; OS = overall survival; mo = month; nr = not reached.

chemotherapy and as first-line treatment in combination with paclitaxel for HER2-positive MBC. The use of trastuzumab as first-line therapy in this setting has changed the natural course of HER2-positive MBC.

Trastuzumab has been investigated in combination with a wide variety of anticancer agents. The addition of trastuzumab to docetaxel significantly improved median overall survival and time to disease progression compared with docetaxel alone [20] (table 1). Trastuzumab in combination with other chemotherapy agents including gemcitabine and vinorelbine has achieved response rates of 40–60% in patients with HER2-positive tumors [21–24]. Triple combinations of trastuzumab, a taxane (e.g. paclitaxel or docetaxel), and a platinum agent, have also demonstrated clinical benefit in first-line treatment of MBC [25–27]. The addition of carboplatin to trastuzumab and paclitaxel improved efficacy compared with trastuzumab plus paclitaxel in patients with HER2-positive MBC [27] (table 1). However, in the BCIRG 007 trial, no significant difference in response rate or overall survival in the trastuzumab plus docetaxel arm compared with the trastuzumab plus docetaxel and carboplatin arm was observed [28].

Cross-talk between the estrogen- and HER2-signaling pathways provides the rationale for targeting these pathways simultaneously in tumors that are both HER2- and estrogen receptor-positive. Trastuzumab has been assessed in combination with the hormonal therapies including letrozole and anastrozole (table 1). The clinical benefit rate observed with letrozole plus trastuzumab was 52% [29], and in the TAndEM trial, the addition of trastuzumab to anastrozole resulted in a clinical benefit rate of 42.7% compared with 27.9% for anastrozole alone [30].

Trastuzumab Side Effects

Trastuzumab is generally well tolerated, with a low incidence of chemotherapy-associated adverse events [17–20]. Infusion-related events such as fever are reported but these are usually mild and limited to the initial infusion. Trastuzumab-associated cardiac dysfunction was first reported in the phase III trial of patients with metastatic disease [19]. The incidence of asymptomatic and symptomatic cardiac dysfunction was 27% in patients receiving doxorubicin and cyclophosphamide (AC) and trastuzumab, 8% for AC alone, 13% for paclitaxel and trastuzumab, and 1% for paclitaxel alone [31]. In this trial, the incidence of symptomatic cardiac dysfunction was 16% based on New York Heart Association criteria in patients receiving trastuzumab with concomitant AC, and 2% in patients receiving paclitaxel plus trastuzumab [32]. However, when combining epirubicin/cyclophosphamide with trastuzumab, no clinically relevant cardiotoxicity was observed [33]. It seems that optimizing clinical management and regimes for patients receiving trastuzumab will help to reduce cardiac side effects [34].

Resistance against Trastuzumab and Clinical Strategies for the Treatment of Patients with Resistance

Although the combination of chemotherapy and trastuzumab prolongs the survival of women with advanced HER2-positive disease, the majority of women develop resistance to trastuzumab. Anticancer activity and synergistic interactions with various chemotherapies provide a rationale for using trastuzumab beyond progression [35, 36]. Trastuzumab has been used effectively in successive lines of treatment, with substantial response rates in patients receiving a second trastuzumab-based regimen [35, 37–39]. To prospectively evaluate the effect of continued trastuzumab treatment after progression, a randomized trial compared either capecitabine alone or with continued trastuzumab treatment. Median times to progression were 5.6 months in the capecitabine group and 8.2 months in the capecitabine-plus-trastuzumab group. Overall response rates were 27.0% with capecitabine and 48.1% with capecitabine plus trastuzumab. Continuation of trastuzumab beyond progression was not associated with increased toxicity [40]. However, the optimal strategy for patients with progression during trastuzumab treatment is not clear since also a change of anti-HER2 therapy, e.g. to the tyrosine kinase inhibitor lapatinib, provides a clinical benefit for patients [41]. Therefore, study results comparing different anti-HER2 approaches in patients progressing on trastuzumab are urgently needed. In this context, it will be of great importance to better understand molecular mechanisms that contribute to trastuzumab resistance. These issues are also important for the identification of novel therapeutic targets with the goal of increasing the magnitude and duration of response to trastuzumab-based treatment.

The benefit of first-line trastuzumab for MBC after trastuzumab therapy in the adjuvant setting is under investigation in the phase II RHEA study of trastuzumab with or without a taxane for first-line MBC treatment after progression on a trastuzumab-based therapy for early breast cancer.

Novel Antibodies Targeting HER2

Pertuzumab

The humanized recombinant monoclonal antibody pertuzumab offers a new therapeutic option in targeting HER2-positive disease. The antibody is directed against the extracellular dimerization domain II of the HER2 tyrosine kinase receptor, which is distinct from the binding site of trastuzumab [42]. Initially, pertuzumab was found to be a potent inhibitor of HER2, inhibiting heregulin-induced activation of HER2 phosphorylation and cell growth [43, 44]. Subsequently, pertuzumab was found to inhibit HER2 dimerization by sterically preventing the HER2 receptor protein (the most common pairing partner of the HER receptor family) to homo- or heterodimerize with other HER tyrosine kinase receptor

proteins, including HER3 [45]. This is in contrast to trastuzumab which does not inhibit the HER2/HER3 interaction. This inhibition of receptor protein dimerization prevents the activation of HER signaling pathways mediating cancer cell proliferation and survival, respectively [45, 46]. There is a growing body of evidence supporting the pertinent role of HER2/HER3 dimers in breast cancer tumorigenesis and thus the relevance of the ability of pertuzumab to prevent this dimer formation: First, an increased signaling potency of this pairing compared with other HER dimers [47] and second, the HER3 dependence of the PI3K-Akt pathway for HER2 activation. This is in line with preclinical data revealing an excellent activity of pertuzumab independent of HER2 expression levels in breast cancer cell lines [45]. However, in clinical trials for MBC with unselected patients, pertuzumab displayed only modest activity when tumors expressed low levels of HER2 [48]. The underlying biological mechanisms for these observed differences are not understood so far.

Interestingly, there seems to be a synergistic antitumor activity of trastuzumab and pertuzumab [49] in the preclinical setting, which was later translated into clinical benefit in several studies investigating this combination in HER2-positive breast cancer after progression during trastuzumab therapy. The observed response rates were as high as 26% and the clinical benefit rates up to 50%, respectively [50, 51]. Currently, ongoing trials investigate the activity of single-agent pertuzumab as well. First results exhibit a potent activity for the monotherapeutic approach [52]. The antibody is also tested now in the first-line treatment of MBC in a phase III study of pertuzumab and trastuzumab plus docetaxel versus placebo and trastuzumab plus docetaxel in patients with previously untreated HER2-positive MBC. In addition, a phase II randomized trial investigates the neoadjuvant combination of pertuzumab and trastuzumab in patients with locally advanced, inflammatory or early-stage HER2-positive breast cancer. So far, there is no evidence for HER2-mediated increased cardiac toxicity by pertuzumab in the larger trials, even if one initial small study with 11 patients reported asymptomatic slight decreases in cardiac ejection function and one congestive heart failure [53].

T-DM1

A further innovative approach to HER2 targeting by specific antibodies is the application of antibody/toxin conjugates. The therapy principle is given by the targeted intracellular delivery of potent antitumor agents via a highly specific monoclonal antibody. Trastuzumab-DM1 (T-DM1, Genentech) consists of the trastuzumab antibody conjugated to DM1 which represents a maytansine derivative [54] and binds to HER2 with an affinity similar to trastuzumab. After binding to HER2, T-DM1 is internalized and DM1 is subsequently released into the cell, thus delivering chemotherapy directly to cells overexpressing HER2. DM1 leads to cancer cell death by inhibiting assembly of microtubules [13]. T-DM1 has shown encouraging

preclinical and early clinical anti-tumor activity with efficient growth inhibition of trastuzumab- and lapatinib-sensitive as well as resistant breast cancer cell lines and tumors with only limited toxicity [54].

One trial investigated the activity of T-DM1 in a multi-institutional, open-label, single-arm phase II study. All 112 patients had progressed on HER2-directed therapy and had received chemotherapy in the metastatic setting. T-DM1 was administered at 3.6 mg/kg intravenously (IV) every 3 weeks. Interestingly, 55% of patients had already been treated with lapatinib after trastuzumab failure. The most common grade 3–4 toxicity was thrombocytopenia (7.1%), no grade 3 or higher cardiac toxicity was reported. With a median follow-up of 4.4 months, the objective response rate was 39.3%. Activity for lapatinib-pretreated patients was consistent in the entire study population with an objective response rate of 38.3%. Among the subgroup of patients who had a follow-up ≥ 6 months, the confirmed response rate (response determined on two different occasions ≥ 4 weeks apart) was 38.2% reflecting the promising activity of this novel therapeutic approach in this heavily anti-HER2-pretreated population [55].

VEGF as Angiogenetic Factor in Breast Cancer and Bevacizumab as Antibody-Based Approach for Targeting VEGF

Angiogenesis is supposed to play an important role in tumor growth and dissemination, and inhibition of tumor-derived angiogenesis is of high interest in oncologic research. Bevacizumab (Avastin[®], Roche Pharma AG) is a humanized monoclonal antibody that binds to the vascular endothelial growth factor (VEGF) family member VEGF-A, reducing the availability of the VEGF as ligand for the VEGF receptors (VEGFR-1 and VEGFR-2). The results of several clinical trials suggest that a synergistic interaction occurs between chemotherapy and bevacizumab. A potential mechanism is the ‘normalization’ hypothesis. This model describes that anti-VEGF agents can cause a transient vasoconstriction of the large aberrant blood vessels in tumors, which may improve blood flow and decrease hypoxia within the tumor allowing better delivery of chemotherapeutic agents.

Clinical Trials with Bevacizumab in MBC

The initial phase I–II trial of bevacizumab in breast cancer was a single-agent dose escalation study in 75 patients with previously treated MBC [56] (table 2). Patients received bevacizumab in doses of 3 mg/kg, 10 mg/kg, or 20 mg/kg administered every 2 weeks. The overall response rate at the 10-mg/kg dose ($n = 41$) was 12%, including 2 patients with a complete remission.

Based on these preliminary data, a phase III randomized trial was undertaken to evaluate bevacizumab in women with

Table 2. Examples of studies with bevacizumab in metastatic breast cancer

Trial design	Phase	ORR, %	TTP, median, mo	OS, median, mo
Bevacizumab dose-escalation 3, 10 or 20 mg/kg (n = 75) [56]	I/II	9.3	5.5	–
Capecitabine plus bevacizumab vs. capecitabine (n = 462) [57]	III	19.8/9	4.86/4.17	15.1/14.5
Paclitaxel plus bevacizumab vs. paclitaxel alone (n = 722) [58]	III	36.9/21.2	11.8/5.9	26.7/25.2
Docetaxel plus bevacizumab (7.5 or 15 mg/kg) vs. docetaxel plus placebo (n = 736) [60]	III	55 or 63/44	8.7 or 8.8/8	83 or 78/73 ^a
Capecitabine plus bevacizumab vs. capecitabine plus placebo/taxane/anthracycline plus bevacizumab vs. taxane/anthracycline plus placebo (n = 1,237) [61]	III	23.6/35.4 37.9/51.3	6.2/9.8 8.3/10.7	21.2/29 23.8/25.2

^a1-year OS rate.

ORR = Overall response rate; TTP = time to progression; OS = overall survival; mo = month.

heavily pretreated MBC. In total, 462 patients were randomized to receive bevacizumab plus capecitabine or only capecitabine [57] (table 2). The combination of bevacizumab with capecitabine resulted in a near doubling of the response rate (19.8 vs. 9.1%; $p < 0.001$). However, the primary endpoint of that trial, progression-free survival (PFS), was statistically identical in the two arms (4.2 vs. 4.9 months). A possible explanation for this result was that with the amount of prior treatment given to participants in this trial, the vasculature of the tumors was more established, and therefore strategies aimed at VEGF and blockade of these proangiogenic signals were less likely to be effective. This explanation resulted in the evaluation of bevacizumab in first-line treatment of MBC. In the ECOG 2100, weekly paclitaxel was administered. A doubling of the response rate (36.9 vs. 21.2%) and almost a doubling of the PFS (median 11.8 vs. 5.9 months) was observed. The final analysis of this study, however, failed to show an improvement in overall survival [58] (table 2). More recently, an updated report about the results of this study with an independent review of the radiology assessments was published with very similar results [59]. The bevacizumab/paclitaxel combination was approved in many countries for first-line treatment of MBC. Another important study in the first-line metastatic setting is the AVastin And DOcetaxel (AVADO) trial. Unlike E2100, this was a double-blind, placebo-controlled study which examined adding two different doses of bevacizumab (or a placebo) to every-3-week docetaxel. PFS was improved by less than 1 month with bevacizumab, a difference which was statistically significant but less impressive than the ECOG 2100 results. Again, no survival benefit for bevacizumab has been seen (table 2). As progressing patients on placebo were allowed to cross over to bevacizumab, it is unlikely that a survival benefit could have been seen with bevacizumab in this study. It is unknown whether the results are a reflection of less synergy between docetaxel and bevacizumab, a smaller antiangiogenic effect with docetaxel dosed every 3 weeks as compared to weekly paclitaxel, or other factors [60]. To examine the effect of different chemotherapeutic regimens, the RiBBOn-1 study was initiated. Regimens in Bevacizumab

for Breast Oncology (RiBBOn-1) is an international randomized phase III trial comparing first-line chemotherapy for MBC (capecitabine, taxane, or anthracycline combinations) with or without bevacizumab. The addition of bevacizumab to either chemotherapy improved overall response rates and PFS with again no difference seen for overall survival [61]. Up to now, these results cannot help to identify the optimal chemotherapy partner for the combination with bevacizumab. The combination with the aromatase inhibitor letrozole is also feasible [62].

It seems that patients have a benefit from continuing bevacizumab until progression when chemotherapy is stopped [63]. The potential benefit of continuing bevacizumab throughout multiple lines of treatment is currently unclear. A number of adjuvant studies with bevacizumab are ongoing. This may be the most efficacious setting for bevacizumab, as the blood supply of micrometastasis is much less established as examined with visible metastatic disease and should theoretically be more sensitive to blockade.

Predictive Factors for Treatment Efficacy of Bevacizumab

There is a lack of predictive markers for the efficacy of anti-VEGF therapy. Furthermore, the predictive markers, VEGF levels in the tumor and blood and VEGFR expression in the tumor, do not correlate with response to anti-VEGF therapy. More recently, however, Schneider et al. [64] demonstrated an important link between single-nucleotide polymorphisms (SNPs) in VEGF and response to bevacizumab utilizing the ECOG 2100 study cohort. The authors reported significantly improved overall survival rates in patients receiving bevacizumab, who carried the specific genotypes, with an additive effect for each allele. However, these findings need to be confirmed in prospective studies. In other tumor entities, retrospective analyses have suggested that the development of hypertension is predictive for therapy outcome. However, in the AVADO trial, no such correlation was observed [65].

Side-Effects Associated with Bevacizumab

The adverse events associated with bevacizumab include hypertension, proteinuria, thromboembolism, impaired wound healing, bleeding, perforation, reversible leukoencephalopathy syndrome, skin rash and infusion-related hypersensitivity reactions [66]. Patients should be monitored for these events throughout the course of bevacizumab therapy. Hypertension is by far the most common adverse event associated with bevacizumab. Blood pressure should be routinely monitored, and hypertension should be medically managed with antihypertensive drugs as deemed appropriate during bevacizumab therapy. Bevacizumab should be discontinued for new life-threatening venous or arterial thromboembolism. To minimize the risk of bleeding or impaired wound healing, bevacizumab should be started at least 4 weeks after surgery or discontinued for at least 6–8 weeks before elective surgery [67]. It seems that with increased knowledge about these side effects and their management, the incidence of severe side effects is decreasing, with a much lower rate reported in the more current trials.

Other Targets for Antibody-Based Approaches

The insulin-like growth factor 1 receptor (IGF-1R) and its associated signaling system is a field of interest as a novel

therapeutic target in cancer. It seems possible that one of the alternative pathways is tumor growth activation via this mechanism. Therefore, different approaches interfering with IGF signaling are currently in development, including several antibodies (e.g. CP-751,871, AVE1642/EM164, IMC-A12, SCH-717454, BIIB022) [68].

Although the epidermal growth factor receptor (EGFR) seems to be involved in mechanisms of malignant growth deregulation such as endocrine resistance [69], no clinical relevant benefit of antibody-based therapies against the EGFR was demonstrated in breast cancer so far. Such approaches might be a part of combined use with several targeted agents [70]. The fact that increased angiogenesis was observed in tumors with HER2 overexpression and a clinical activity observed of the combined use of trastuzumab and bevacizumab in MBC, is the rationale for the use of both antibodies in ongoing clinical trials.

Conflict of Interest

Volkmar Müller has received research funding by Aventis Pharma and Roche Pharma, speaker honoraria by Aventis Pharma, Amgen, Pfizer Pharma, Novartis, and Genomic Health. Isabell Witzel has no conflict of interest to declare. Elmar Stickeler has received research funding by Aventis Pharma, speaker honoraria by Aventis Pharma, Amgen, Novartis, and Roche Pharma.

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