In the Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial designed in 2005, four agents against defined molecular targets were assigned to metastatic lung cancer patients based on predefined biomarkers. In this trial, it was demonstrated that a prospective biomarker-driven personalized therapeutic approach in lung cancer is feasible. In this article, preliminary results of this trial as well as the concept of biomarker-driven therapy of non-small-cell lung cancer patients will be summarized. As one of the results from this trial, re-biopsies of metastatic disease will become increasingly important and should be performed with acceptable risks for the patient, and combined with treatment options, whenever possible.

Key-terms: EGFR • lung cancer • metastatic disease • personalized medicine • sequential biopsies

Lung cancer remains one of the most frequent human cancers and the leading cause of cancer-related deaths in the USA with a 5-year survival of only 15% [1]. The vast majority of lung cancer cases (~80%) are non-small-cell lung cancers (NSCLCs) and the remaining fraction are small-cell lung cancers. At diagnosis, approximately 40% of NSCLC patients present with metastatic disease. In this stage, systemic treatment is usually performed and considered as a palliative situation. However, the course of this disease is only transiently improved by therapeutic efforts [2]. Even though categorized into various morphological subtypes, treatment with chemotherapy is more or less uniform and not specifically adapted.

Besides chemotherapy, a variety of novel strategies that specifically inhibit signaling pathways involved in tumor growth and progression have been studied in metastatic NSCLC. Among them, substances inhibiting the activity of various receptor tyrosine kinases (RTKs) have gained much attention, including small molecules blocking the intracellular ATP binding site of the receptor and neutralizing antibodies targeting the extracellular ligand-binding domain. Moreover, antibodies specifically directed against various growth factors have been constructed, thereby also affecting the activity of RTKs in an indirect manner.

To date, two strategies have been adopted into the general treatment of NSCLC in defined settings: tyrosine kinase inhibitors (TKIs) of the EGF receptor (EGFR) family and combination of anti-VEGF therapy with conventional platinum-based therapy. A host of further agents are in clinical or preclinical development. The targets of these novel agents include the inhibition of growth factor receptors, such as EGFR and IGF-receptor I (IGF-IR), several intracellular signaling pathways, such as PI3K, and the tumor vasculature via inhibition of angiogenic receptors or pre-existing vessels by a mechanism not yet fully understood (e.g., vascular disrupting agents). However, a substantial amount of clinical Phase II and III trials failed to demonstrate a significant benefit in overall survival in unselected patients. Hence, an intensive search for predictive markers to preselect patients has begun.
Predictive markers

Currently, efforts on further molecular analyses of different phenotypes of NSCLC (in particular adenocarcinoma) have been made. The extension and exploitation of molecular alterations help to improve the categorization of NSCLC and provides the opportunity to allocate targeted treatment to specific molecular characteristics. As a hypothesis, defined predictive markers such as baseline values or changes in the value of the marker over time assessed by a defined clinical end point should forecast the efficacy or toxicity of treatment with targeted agents [1,2].

To date, EGFR mutations within the tyrosine kinase domain (exons 18–21) are the only markers for selecting EGFR TKI therapy over chemotherapy in therapy-naive metastatic NSCLC [4]. However, several preclinical and clinical studies suggest that these mutations do lead to differential response in dependence on the precise type of mutation and the localized exon. Besides EGFR mutations, multiple other potential predictive markers have been investigated (reviewed in [5]). In selected studies, some markers have been correlated to improved outcome when treated with EGFR TKIs, such as EGFR amplification/high polysomy or high EGFR staining intensity performing immunohistochemistry; however, these results did not prove to be consistent in other studies [6–8]. Still, the latter parameters have not been incorporated into clinical routine since there were also several studies with negative findings and prospective studies showing benefit from preselection based on these criteria are pending. Moreover, the efficacy of EGFR targeting drugs could be influenced by several determinants including the shift of heterodimer formation pattern (with other HER family members) or further gene alterations both leading to constant receptor overactivation, overexpression of other growth factor receptors (e.g., IGF-1R), and alterations of activated signaling pathways downstream of EGFR [9]. For example, these downstream signaling pathways may be constantly activated by K-RAS mutation or loss of PTEN, leading to clinical failure to EGFR TKIs.

Targeted therapy & resistance

At least in the USA, two small molecular weight TKIs against the EGFR, gefitinib (in particular clinical settings) and erlotinib, have been approved for single-agent therapy of relapsed NSCLC patients with metastatic disease. In Europe, gefitinib has been only approved for the treatment of NSCLC patients harboring EGFR mutations. Moreover, antiangiogenic agents have been integrated in therapeutic concepts. In two large clinical trials, an anti-VEGF antibody (bevacizumab) has demonstrated efficacy in combination with a platinum-containing chemotherapy in patients with advanced NSCLC of non-squamous histology [10,11].

EGF receptor

Several clinical studies have demonstrated that the treatment of unselected NSCLC patients with gefitinib or erlotinib led to only moderate improvement in overall survival (BR21: median of 6.7 months for erlotinib in second-line therapy of metastatic NSCLC versus 4.7 months for placebo; INTEREST trial: non-inferiority for gefitinib versus docetaxel in platinum-based chemotherapy pretreated NSCLC with overall survival of 7.6 vs 8.0 months) [6,8]. However, some patients do not show response to EGFR TKIs that may be characterized by other somatic mutations in genes, such as K-RAS, which also have an impact on the EGFR signaling pathway or by mutations in the EGFR gene that are not associated with sensitivity to EGFR-TKIs [5,7]. In addition, patients with initial response often experience acquired resistance while being on therapy. Results from early clinical studies on limited patient numbers implied that approximately 50% of NSCLC resistance to TKI may be due to the occurrence of a secondary mutation in exon 20 of EGFR (T790M) and 20% to c-MET amplification [12]. Additional genetic changes may also occur. Interestingly, most trials demonstrated dramatic response to EGFR TKIs for subgroups of patients [13]. Besides clinical characteristics (e.g., female gender, predominant adenocarcinoma, no smoking history and Asian background), these patients have been linked to the occurrence of somatic sensitizing mutations in the EGFR leading to an initial clinical response of 60–80%. Moreover, EGFR mutations seem to be a prognostic factor and are associated with a favorable outcome with both EGFR TKI treatment and conventional platinum-based chemotherapy (reviewed in [14]).

K-RAS/B-RAF

Upon activation of EGFR, several pathways may be involved in downstream signaling. K-RAS belongs to the RAS family of genes (K-RAS, N-RAS and H-RAS) and is an important effector of ligand-bound EGFR, mainly but not exclusively signaling through B-RAF and the MAPK axis. In turn, B-RAF is one of three members of the RAF kinase family (A-RAF, B-RAF, C-RAF) and links these signaling molecules to downstream proteins of the MAPK family, which control cell proliferation and differentiation [15,16]. Besides the MAPK family, EGFR signaling also involves the PI3K protein family, which is the key mediator between growth factor receptors and intracellular downstream signaling pathways leading to proliferation, differentiation and inhibition of apoptosis [17]. However, cross-links between MAPK and PI3K signaling pathways exist.
Angiogenesis

Angiogenesis is a complex process regulated by cellular cues, multiple receptor-mediated signaling networks, and a number of pro- and antiangiogenic factors. Since VEGF is considered the predominant angiogenic factor in tumors, anti-VEGF strategies have been developed. Although bevacizumab is the only antiangiogenic agent currently approved for treatment of NSCLC, many others are in clinical trials, including various multi-TKIs. So far, the clinical benefit of these agents in combination seems to be somewhat limited. Similar to resistance to EGFR TKIs, both pre-existing and adaptive resistance mechanisms have been postulated leading to failure of antiangiogenic drugs.

However, both phenomena may underlie similar molecular and cellular mechanisms such as upregulation of alternative proangiogenic signals, protection of the tumor vasculature either by recruiting proangiogenic inflammatory cells or by increasing protective pericyte coverage and accentuated invasiveness and metastatic seeding of tumor cells. Apart from histology, no tools are available to guide the use of antiangiogenic agents. As a result, these therapies are used by exclusion only. Several candidate biomarkers have been investigated in NSCLC as predictive indicators of response to antiangiogenic therapy. Some of these biomarkers include measures of angiogenesis itself, such as visual quantification of microvessel density, and more recently, evaluation of gene signatures or expression levels at the mRNA or protein level.

Retinoid X receptor

Retinoid receptors, which play a critical role in cellular growth modulation, division, induction of differentiation, and activation of apoptosis, have been investigated as potential targets for novel cancer therapies for several years. Retinoids, including vitamin A and its analogues, regulate the growth and differentiation of a wide variety of cells. Retinoids act by binding to two classes of intracellular retinoid receptors: retinoic acid receptors and retinoid X receptors (RXRs), each with three subtypes, α, β, and γ. The expression of many retinoid receptors, including RXRβ, is reduced in some NSCLC biopsy specimens and increased RXRβ expression has been associated with an increased survival in NSCLC patients.

Although in advanced NSCLC patients a Phase III clinical trial failed to meet its primary end point of prolonging overall survival with the combination of first-line platinum-based chemotherapy and retinoid bexarotene, a yet to be defined subgroup of patients did benefit from the addition of bexarotene.

The BATTLE program

Design

As an example for the development of a personalized therapeutic approach in patients with advanced NSCLC, the prospective Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) program has been initiated. The design of this pioneering Phase II trial was reported at the American Association for Cancer Research (AACR) 2010 annual meeting. The aim of this program was to prove the feasibility of selecting patients with key pathway variations and including these patients to appropriate clinical trials. Patients with diagnosed NSCLC and prior chemotherapy treatment were re-biopsied in order to obtain enough frozen tumor tissue, not only to assess the current status of various biomarkers, but also to enable later laboratory analyses. Tissue was obtained by performing trans-thoracic needle aspirations guided by CT scans. Moreover, serum from all patients was collected. In the first step (BATTLE-I study), designed in 2005, 11 biomarkers reflecting the activation status of promising pathways were routinely assessed. In brief, five different biomarker groups were defined: EGFR positive (evidence of EGFR mutation, increased EGFR copy number or high EGFR polysomy), K-RAS/B-RAF positive (evidence of B-RAF or K-RAS mutation), VEGF positive (expression of VEGF or VEGFR2), RXRs/CYCLIN D1 positive (expression of RXRα, RXRβ, RXRγ or CYCLIN D1 expression or CYCLIN D1 amplification) or negative for all markers. Protein expressions of the selected markers were evaluated with immunohistochemical staining while amplification and mutational analyses were done performing fluorescence in situ hybridization and PCR with subsequent sequencing, respectively. In addition to this umbrella study, a panel of four Phase II studies was initiated treating patients with previously treated, advanced NSCLC, with either erlotinib (Tarceva®; Roche), sorafenib (Nexavar®; Onyx/Bayer), vandetanib (Zactima®, AstraZeneca) or erlotinib plus bexarotene (Targretin®; Eisai). The initial group of patients was equally randomized to receive one of these four treatments.

Once enough baseline results had been collected, the data from this group were used to assign the remaining patients to the treatment arms performing an adaptive Bayesian statistical method based on each patient’s biomarker profile. With the Bayesian statistical method and a selected biomarker ranking taking precedence, the likelihood for patients to receive the appropriate treatment should be increased. All patients stayed on medication until progression; however, the primary end point was disease control at 8 weeks of treatment.
Collectively, this BATTLE program not only tried to set-up a first approach to personalized medicine but also to validate the concept of predictive markers. After completion of treatment, the additional frozen biopsy and serum samples will be used to explore the genomic and proteomic profiles using gene and cytokine arrays. These data will be correlated with the patient’s outcome in order to identify novel predictors of therapy response or resistance. The final goal of this study is the set-up of well designed Phase III trials, thereby reducing costs.

■ Preliminary results

Biopsy procedures as performed by trans-thoracic needle aspirations were well tolerated with an 11.5% pneumothorax incidence (mainly grade 1/2). Study treatments were generally well tolerated and consistent with known toxicities from prior studies. Some data have been presented at the AACR and American Society of Clinical Oncology (ASCO) annual meetings 2010 and will be briefly reflected in the following [32]. Until stopping recruitment in October 2009, 341 patients were enrolled. A total of 255 patients were randomized to one of the four treatment groups using the statistical methods of equal randomization for the first 97 patients followed by adaptive randomization for a further 158 patients (Figure 1). Equal randomization distributed patients to a respective treatment based on a prestudy set-up allocation table while adaptive randomization modified this initial allocation table based on early results from the BATTLE trial. With 244 patients finally evaluable, the disease control rate at 8 weeks as the primary end point of this study was 46% across all treatment groups. Patients were treated with erlotinib (n = 58), vandetanib (n = 52), erlotinib/bexarotene (n = 36) and sorafenib (n = 98), and had a disease control rate at 8 weeks of 34, 33, 50 and 58%, respectively. The median overall survival was 9 months (1 year survival: 38%). Interestingly, patients who were able to achieve disease control at 8 weeks had a significantly better survival rate than patients with uncontrolled disease (11.3 vs 7.5 months; p = 0.002).

As expected, erlotinib yielded a better response in patients with EGFR mutations (15% of all patients; p = 0.04). By contrast, sorafenib in patients whose tumors had an activating EGFR mutation yielded a significantly lower disease control rate than for patients without the mutation (23 vs 64%; p = 0.012). Moreover, patients with a high EGFR polysomy and treated with sorafenib had a lower disease control rate at 8 weeks than for those without (27 vs 62%; p = 0.048). Mutations in K-RAS, a downstream mediator of EGFR signaling, were detected in 20% of all patients. Interestingly, these patients tended to respond better to sorafenib with a disease control rate of 61% (n = 18) relative to just 32% for patients with a K-RAS mutation who were treated with any other drug [32]. Patients with VEGFR2 overexpression (40% of all patients) had a poor response to sorafenib compared with a good response to vandetanib (p = 0.05). The erlotinib and bexarotene combination worked best in patients that were positive for CYCLIN D1 or had EGFR copy number amplifications (p = 0.001 and p = 0.006, respectively). Results of the planned gene array analyses have not been presented, yet.

Future perspective

Since there is no full publication of the BATTLE trial, the above described data must be interpreted with caution. Currently, we have no detailed information on most quality criteria such as selection of eligible patients, patient dropouts, adequacy of follow-up and statistical procedures. In particular, early findings such as the good outcome for sorafenib in k-ras-mutated tumors need to be validated in larger confirmatory studies. In contrast to these data, a single-center Phase II trial showed no
correlation of K-RAS mutations with response, progression-free survival, or overall survival in 34 relapsed or recurrent NSCLC patients treated with sorafenib. In this latter study, the patient number was quite low with 11 out of 34 patients being positive for K-RAS mutations (32%). Moreover, the precise mechanisms of the link between the biomarker and the drug effect need to be more closely investigated. As mentioned in the BATTLE trial, extensive mRNA profiling from all frozen tissue samples with gene arrays and cytokine analyses from sera are planned. These investigations are important and may lead to further understanding and new hypotheses.

The most important finding of the BATTLE trial is that biomarker-driven therapy in NSCLC is feasible. This includes that the risks for re-biopsies of the tumors seems to be acceptable for the patient, however, this will be an individual decision for every patient. Hence, not every patient may be eligible for this approach. It should be stressed that in addition to a biopsy before treatment initiation with a molecular defined agent, it would be necessary to also perform another biopsy upon progression under this agent in order to compare genetic and proteomic profiling and to enhance our understanding on resistance mechanisms.

As primary end point of this study the disease control rate at 8 weeks after treatment initiation has been chosen. This end point may enable a quick assessment of therapeutic benefit. However, it indicates that this study at first should be a proof of feasibility. Since all therapies applied in this study are generally given until disease progression, one has to define when to determine the response to the given agent. In contrast to best response, this end point should give a quick indication of response within a defined interval. By contrast, no conclusions to best response, progression-free interval and overall survival can be made. These data should be available with the full publication, which is pending.

Similar to the BATTLE-1 study, further trials have been initiated – the BATTLE-2 trial is intended to enroll 320 patients with K-RAS-mutated NSCLC who have already been treated with chemotherapy. These patients will be randomized to different combinations of erlotinib and inhibitors of the AKT or MEK pathways. Moreover, the BATTLE -3 study will include metastatic NSCLC patients who are therapy-naive combining chemotherapy with biologic agents. Trials for surgically resected patients are also planned. However, although the designs of the BATTLE studies include promising markers for selection of therapy strategies, several challenges remain to improve this concept. Clinical trials that require research biopsies of participants must be well designed (reviewed in [33]). Various ethical concerns must be considered such as assessment of the risk of harm to participants and the adequacy of voluntary informed consent. In particular, biopsies that are performed for research purposes only without clinical consequences may be necessary for enhancing our understanding of resistance mechanisms but, in turn, must be in consent with ethical regulations. In any case, the patient must be extensively informed including risks, rationale and requirements of the study, as well as of treatment alternatives, minimizing the risk for misunderstanding. To address these questions, the Cancer and Leukemia Group B Ethics Committee proposed guidelines for clinical trials involving mandatory research biopsies [33].

Most importantly, there is a clear need for the inclusion of biomarker programs into clinical trials to identify and verify additional possible predictive markers. Moreover, the technology used to measure various biomarkers needs to be standardized including selection of optimal methodology and interpretation of the data that are generated by these methods [33]. In addition, the selection of the appropriate clinical trial design for biomarker identification and validation is mandatory as described [34,35]. Finally, one characteristic of most clinical studies was that biomarker investigations were performed on a rather small percentage of the patients included in a large series, which questions the representativeness of the findings.

Still, increasing data from clinical lung-cancer trials (such as IPASS) support the hypothesis that identifying selected subgroups of patients might particularly benefit from various TKIs, improving overall and progression-free survival [36,37]. With testing for EGFR mutations in stage IV lung-cancer patients, the biomarker-driven therapy selection has become reality. Recently, further promising targets have been evaluated. For example, a novel target that has been detected in some lung cancer patients is EML4-ALK (echinoderm microtubule-associated protein-like 4 fused to the intracellular kinase domain of anaplastic lymphoma kinase) [38,39]. A Phase I/II clinical trial in 31 NSCLCs demonstrated a 65% response rate with treatment of the specific ALK inhibitor crizotinib [40]. Interestingly, patients who harbor this mutation do not benefit from EGFR TKIs [41]. Further studies are ongoing [42]. However, in unselected NSCLC patients, the occurrence of EML4-ALK seems to be a rare event with a low prevalence ranging from 1.5 to 6.7% [41,43]. This low frequency is increased in younger patients, never/light smokers, males and adenocarcinoma patients.

**Conclusion**

The BATTLE trial has demonstrated that individualized selection of a broader spectrum of targeted agents based on predictive markers seems to be feasible. This includes the need for re-biopsies to be able to study the
present marker status in the respective tumor. For the patient, the re-assessment of tissue marker would imply an additional bronchoscopy or computed tomography scan-guided biopsy. Most importantly, tissue biopsies should be obtained with sufficient quantity and quality. Finally, with results from trials such as the BATTLE trial, new hypotheses may be generated that will be tested in further clinical trials. This may lead us closer to the ultimate goal, to treat our patients with a greater chance of success and to decrease the mortality and morbidity of lung cancer patients.

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Executive summary
- The Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial has demonstrated:
  - Prospective biomarker-driven personalized therapeutic approach in lung cancer is feasible.
  - Sorafenib did well in patients with K-RAS mutations (preliminary data).
- What needs to be done:
  - Full publications of the trial and ongoing genetic and proteomic profiling are pending.
  - Re-biopsies of patients may enable individualized therapy selection but this potential benefit has to be balanced against risks of additional bronchoscopy/CT-guided needle punctation.
  - Further studies with similar design are needed to better understand tumor biology and resistance mechanisms.

Bibliography
Papers of special note have been highlighted as:
- of interest
- of considerable interest

Concise and recent review on tumor angiogenesis.

Outstanding overview on mechanisms of anti-VEGF-directed agents.
Personalized therapy in the BATTLE Review: Clinical Trial Outcomes


Introduction and overview on current concepts of predictive markers for antiangiogenesis.


Important overview of clinical trial designs.


Full publication of the IPASS study analyzing the concept of personalized therapy in adenocarcinoma.


