Targeted therapies; who detects the target?

After decades in which anti-cancer drugs mainly were designed to inhibit tumor cell proliferation or to interfere with DNA integrity in order to induce apoptosis in a very general way, we recently have been witnessing the development of a new class of anticancer drugs specifically targeted at biological properties of tumor cells. Examples are Imatinib working against the Kit oncogene and Bcr-Abl tyrosine kinase, respectively, Trastuzumab against Her2Neu, and several drugs including Gefitinib and Erlotinib against EGFR [5,6,11]. In addition, drugs targeted against VEGF, SRC and other molecules have been registered or are in development [4,12]. The concept of targeted anti-cancer drug therapies is very appealing and preclinical studies in this area have been very promising. Yet, results in some clinical studies have been less exciting than had been expected on the basis of pre-clinical studies [8].

Two factors are relevant in this respect. The first is whether in the tumors of the patients receiving these therapies, the target molecule actually plays a critical role. One approach to determine this is trial and error. Just give the drug to any patient with a tumor type in which some activity of the drug has been reported. This approach has a number of drawbacks. First of all, treating a patient with a drug that has no chance of being effective because it does not match with the biology of that tumor, is a waste of time in which alternative drugs with a higher chance of response could have been administered to the patient. Second, costs of these targeted drugs have a major impact on healthcare budgets and hardly justify a trial and error approach [16]. The other approach is prediction of response to therapy, based on demonstrating certain biological characteristics of the target molecule in a given tumor. This frequently implies immunohistochemical analysis of gene expression in tumor tissue samples. This approach has proven to be of value in the case of c-kit and Her2Neu. Yet, standardization issues remain, both in terms of performing as well as interpreting the stainings. In this respect looking at DNA alterations like amplifications seems to be more robust, and FISH actually now is the gold standard for analyzing Her2Neu amplifications.

The second factor is whether in addition to the status of the target gene, other major biological tumor characteristics determine clinical behavior in such a way that the effect of targeted therapy is futile. Diagnostic tests that look at the status of the target gene only – like FISH and immunohistochemistry – may give a false prediction of clinical outcome. Genome wide approaches like expression microarrays or array CGH could be more informative here, but this area is largely unexplored [18].

Also in diagnostic pathology we need to find new ways of anticipating this issue. Most tumor classifications today are based on the organ of origin and on similarity in microscopic phenotype. To some extend, expression of certain proteins measured by immunohistochemistry, is already used for classification purposes, especially in lymphomas. Yet, most of the time these are different proteins than those to which the drugs are pointed. Since drugs work best on tumors to whose biology they match, it seems justified to aim for tumor classifications that are based on prediction of response to (targeted) therapies. While this is a revolution in diagnostic pathology, it also holds a paradigm shift in the practice of randomized clinical trials (RCT). The variables in randomized clinical trials are no longer only (or mainly) the different drug regimens that are compared, but rather the combination of the diagnostic tests and drugs. In fact this increases complexity of the RCT in that not only methodological aspects of drug therapy (dosage, pharmacokinetics, etc.) matter, but also methodological aspects of the diagnostic test.

Are we ready for this new situation? Which test should be used in combination with which drug? How do we overcome the issue of other biological tumor properties overruling the response prediction based on assessment of activity of the target molecule in a patient’s tumor. Standardization issues are important here and technical platforms, thresholds, and reproducibil-
ity need to be considered [1,7,10,15]. Strategies for developing and implementing a diagnostic test have been described and ideally include retrospective studies, in house prospective studies, multi-center prospective studies and ultimately a randomized clinical trial to ultimately prove the benefit for the patient [2]. Not unlike drug development, test development and implementation is a process that takes many years. This approach carries the risk that by the time a test has been fully validated, it has become obsolete because newer, more fancy methods have become available, that sometimes enter the market with less extensive validation [3,17]. Since pathology is a discipline with thousands of diagnostic tests with largely undocumented sensitivity, specificity and predictive value, this is a realistic option. At the moment, it seems that test development is not initiated before a drug is enters a phase three clinical trial. This can lead to insufficiently validated tests being implemented in diagnostic practice. In addition to the fact that a test can get less appealing by the time it is fully validated, also the combination of test and drug can become outdated because newer drugs or protocols have emerged, and the process can start all over again. A work around for this pitfall could be to use unsupervised biological classifications for stratification of patient groups, which intrinsically are more stable than supervised drug response based classifications.

Is there a way out? Before a patient is included in a new therapeutic trial, ideally it would first have to be established whether the tumor cells are intrinsically sensitive to the new therapeutic agent. The most straight forward approach is to isolate tumor cells from a diagnostic biopsy and incubate these cells with the active compound. This strategy is feasible in those tumors in which isolated tumor cells are easily obtainable, in particular leukemia’s and to a lesser extent lymphomas [14]. Before starting large randomized trials it is possible to test in a restricted number of patients whether such a functional assay indeed accurately can predict clinical response to the new drug. The randomized trial can than be restricted to only those patients whose tumor cells were shown in vitro to be sensitive. For epithelial tumors this approach is more difficult because of problems with obtaining isolated tumor cells for in vitro testing. Cell line models are used as substitute, but these cell lines result from very stringent in vitro selection for an immortal phenotype and thus are unlikely to reliably reflect the sensitivity for different therapeutic agents of the originating tumor cells.

An alternative could be in silico sensitivity testing. If one knows the (putative) mechanism behind the cell death inducing effect of the new drug on test, knowledge of expression levels of all involved genes (e.g. obtained by micro-array analysis) should be able to accurately predict the sensitivity of a tumor to the tested agent. Such a strategy seems applicable for the treatment of part of chemotherapy refractory diffuse large B-cell lymphomas that are characterized (using micro-array profiling) by constitutive NFkB expression resulting in expression of different anti-apoptotic genes. This group can in principle be treated with an NFkB inhibitor like Bortezomib.

Today, cellular oncology is facing the challenge to translate tumor biology into clinically applicable knowledge, ultimately in the form of well documented diagnostic tests [9,13]. Part of this challenge is to find a way for validating such diagnostic tests more rapidly, than currently is the case. A solution for this dilemma may be to develop highly predictive intermediate endpoints like the in vitro functional essays as mentioned above. Also highly sensitive response monitoring, e.g. by molecular imaging can be of help here. An even more basic approach would be to reconsider the study designs used. Current study designs could actually hamper progress in cancer therapy. Nowadays, early phase clinical trials usually involve advanced stage patients only, in whom only limited effects can be expected. Furthermore, frequently large sample sizes are needed to allow the detection of small effects, but is that what we are after? Wouldn’t it make more sense to use trial capacity for studies that have a chance of finding larger differences in smaller series based on a match between drug mechanisms and tumor biology, instead of including many patients to establish a small difference? It anyway is clear that from now on drug development and test development have to go in parallel.

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