Guest Editorial

Molecularly-Targeted and Biological Anti-Cancer Therapy

(Targeted therapy / biological therapy / biopharmaceutical / biosimilar / tailored therapy / personalized medicine)

P. KLENER JR.1,2, P. KLENER1,2

1Institute of Pathological Physiology and Centre of Experimental Haematology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic
21st Department of Medicine – Department of Haematology, First Faculty of Medicine, Charles University in Prague and General University Hospital, Prague, Czech Republic

The advent of the new millennium was characterized by emergence of dozens of so-called new anti-tumour drugs that differed in many substantial aspects from the established, so far widely used chemotherapy agents. Along with the entrance of these novel anti-tumour agents into clinical practice several new terms and designations came up as well, including molecular targeted therapy, biological therapy, epigenetic therapy, differentiating therapy, gene therapy, and tailored/individualized/personalized therapy. Unfortunately, not only in the daily news, but also in numerous scientific reports the above-given terms and notions either have been interchangeably (which in our opinion they are not), or each time their meaning has been interpreted in a more or less different way. As a consequence a substantial part of the current generation of clinical oncologists, who have had limited personal experience in the field of molecular biology, vainly grope for a simple explanation of how to use these terms properly. For this reason we have decided to set up a brief editorial that would provide an unsophisticated review focused on these new anti-cancer agents and treatment approaches, with special focus on the molecular roots from which they have originally stemmed. We expect that the current article will help ordinary clinical oncologists to gain insight into the molecular basis of the modern treatment anti-cancer strategies.

Targeted therapy

Targeted therapy (using rationally designed, molecularly-targeted therapeutics) is a conceptual notion that should be used for such a treatment approach that has been developed based upon the known pathophysiology and/or biomarker of a particular malignancy. Monoclonal antibodies (e.g. anti-CD20 rituximab) and tyrosine-kinase inhibitors (TKI, e.g. imatinib) were among the first molecularly-targeted anti-cancer agents introduced into clinical practice in the therapy of B-lymphoproliferative disorders and chronic myelogeneous leukaemia (CML), respectively. In our opinion, both rituximab and imatinib represent prototypical examples of the two different molecularly-targeted anti-cancer treatment approaches (Fig. 1). The rituximab approach is based on targeting a biomarker expressed on the surface of cancer cells (CD20 antigen), while the imatinib approach is directed at the druggable molecule involved in the cancer pathophysiology (inhibition of the aberrant BCR-ABL tyrosine-kinase activity).

Rituximab is a chimeric antibody (part human, part murine) used for the therapy of CD20+ B-cell lymphoproliferative disorders. It exerts its anti-tumour activities via several recognized ways, including direct induction of apoptosis, complement-dependent cytotoxicity (CDC) and antibody-dependent cellular toxicity (ADCC) (Reff et al., 1994; Cartron et al., 2004). Besides that, application of rituximab is associated with immunomodulation, as rituximab binds not only CD20 antigens via its Fab fragment, but also Fc-γ receptors expressed on a wide range of immune cells via its Fc fragment. Antibodies can be conjugated to radioisotopes (anti-CD20 conjugated to 90Ytrium ibritumomab tiuxetan) or toxins (anti-CD30 antibody conjugated to antitubulin agent monomethyl auristatin E (MMAE) brentuximab vedotin) (Younes et al., 2010). Antibodies can...
be genetically engineered as bi-specific, e.g. single-chain anti-CD3/anti-CD19 blinatumomab, which redirect CD3-expressing T cells toward CD19-expressing B-cell malignancies.

Imatinib, signal transduction inhibitor 571 (STI 571), belongs to a family of small-molecule tyrosine-kinase inhibitors (TKIs) (Druker 2002, 2004). In case of imatinib, the unveiled pathophysiology of CML (namely the indisputable role of the aberrant BCR-ABL tyrosine kinase signalling) eventually led to testing the agents that would specifically inhibit these oncogenic pro-survival signals. In both of these two prototypical examples of molecularly-targeted therapy (rituximab and imatinib), clear definition of a cancer-specific biomarker or druggable molecule/signalling pathway preceded the subsequent preclinical and clinical testing and successful introduction of the anti-cancer agent into the clinical practice. Molecularly-targeted therapy thus stands in conceptual opposition to non-targeted therapy represented mainly by the conventional chemotherapy drugs (classical cytostatics).

**Conventional chemotherapy drugs**

Conventional chemotherapy drugs have been used based upon empirical data, i.e. based on the preclinical and clinical experience with their anti-tumour efficacy, with limited or no molecular substantiation that would explain how or why these drugs actually exert their anti-tumour activity in a particular malignancy. Classical cytostatics were rarely developed to treat a specific cancer subtype, but rather to treat malignant diseases as such or to treat a broad range of malignant diseases (e.g. haematologic malignancies, solid cancers, etc.). Importantly, testing these agents preceded the recognition of the molecular mechanisms responsible for their anti-cancer efficacy. The first alkylation agents were introduced into clinical practice before the discovery of DNA structure based upon the observations that nitrogen mustard induced long-term cytopoenias in First World War soldiers who had survived gas attacks. Two objections might be raised against the very need to establish the notion “targeted therapy” as a new treatment approach.

First, one might regard the beginning of the targeted therapy era (= introduction of monoclonal antibodies and TKIs into clinical practice) as a mere climax of a gradual process of unfailing deepening of our knowledge of the biology of cancer diseases with no specific boundary to separate the targeted and non-targeted approaches. Second, one might object that the majority of the so-called conventional chemotherapy agents share at least some features with the targeted therapeutics. The antimetabolites “targetedly” interfere with the synthesis of the DNA molecule, alkylating agents “targetedly” block DNA replication, vinca alkaloids “targetedly” inhibit mitosis, etc. We are, nevertheless, deeply persuaded that this is an inadmissible reduction and unification of two conceptually separate treatment approaches. First, the molecular basis of a particular cancer subtype was usually unveiled (if ever) years or decades after the introduction of classical cytostatics into clinical practice. More importantly, most of the conventional chemotherapy agents belong to broad-spectrum anti-cancer drugs that interfere with very basic cellular processes, such as DNA synthesis, DNA replication, transcription, translation, protein degradation, cell division, etc. (Fig. 2).

It must be taken into account that these general cellular processes are in no way limited to cancer cells, but are shared by all (proliferating) cells. This has distinct pros and cons. Conventional chemotherapy agents can be regarded as broad-spectrum anti-cancer agents with
limited differences in their anti-tumour activity among different patients with the same malignancy. Moreover, the same agents are often used for the therapy of very diverse malignancies. As a result, administration of classical cytostatics tends to be associated with considerable toxicity toward healthy tissues. At the molecular level conventional chemotherapy agents do not bind and inhibit aberrantly expressed proteins or activated signalling pathways in order to specifically interfere with the complex biology of cancer cells. On the contrary, anti-tumour activity of the classical cytostatics resides in “mere” inhibition of proliferation (direct anti-proliferative effect) and indirect induction of apoptosis (indirect pro-apoptotic effect), usually secondary to the genotoxic stress or cell cycle arrest. Targeted therapeutics usually interfere with particular molecules and/or aberrantly activated signalling cascades at various levels, which can have very specific consequences for the biology of cancer cells. In contrast to classical cytostatics, molecularly-targeted agents can directly trigger apoptosis, shut down oncogenic signals, inhibit self-renewal, or induce differentiation of cancer cells. Moreover, targeted therapy can also be directed at the non-malignant cells of the tumour microenvironment, and thereby can interfere with complex processes such as angiogenesis, metastasizing, immune response, etc.

Targeted therapeutics usually exert narrow-spectrum anti-cancer activity that is more or less directed at the tumour tissue, i.e. at the cancer cells, and/or “non-malignant” cells of the tumour microenvironment that sustain tumour growth (= on-target effect), while toxicity toward healthy tissues is more or less limited (= off-target effect) (Fig. 3). Anti-CD20 antibody rituximab binds to CD20 expressed on the surface of malignant lymphocytes (on-target effect), and it binds and eliminates non-malignant CD20+ B cells as well (off-target effect). Besides that, rituximab also binds Fc-γ receptors of diverse types of leukocytes via its Fc fragment, thereby inducing immunomodulation (off-target effect). Imatinib binds the BCR-ABL fusion protein, thereby inhibiting its aberrant tyrosine-kinase activity (= on-target effect). Besides that, imatinib binds and inactivates cABL of healthy cells, as well as receptor tyrosine kinases cKIT and PDGFR. While imatinib-mediated inhibition of cKIT in patients with CML can be regarded as a clear off-target effect, it represents an on-target effect in patients with gastrointestinal stromal tumours (GISTs) that are characterized by aberrant cKIT activation.

Targeted therapy depends on biomarkers. A biomarker can be any molecule expressed on/by malignant cells or by “non-malignant” cells of the tumour microenvironment that can be used for diagnostic purposes and the expression of which correlates with biologic aggressiveness of the tumour or response to therapy (prognostic/predictive biomarker). However, the molecularly-targeted therapy approach can only be designed based on those biomarkers that represent druggable targets. For example, the increased level of lactate dehydrogenase (LDH) is one of the established prognostic biomarkers in non-Hodgkin lymphomas. LDH, however, does not represent a druggable target. In contrast, the expression of VEGF by tumour cells and non-malignant cells of the tumour microenvironment represents a valuable druggable target for anti-VEGF bevacizumab.

**Biological therapy**

Biological therapy comprises a group of therapeutic agents based on their structure that is derived from the bio-molecules (biological medicinal products, biologi-
Molecularly-targeted anti-cancer agents exert both cytotoxic effects toward malignant cells ("on-target" effects) and non-malignant cells ("off-target" effects). As both on-target and off-target effects depend on the "target" (druggable molecule), the same anti-cancer agent may exert different on-target/off-target effects depending on the cancer subtype. For example, the druggable target of the tyrosine-kinase inhibitor imatinib is different in the therapy of CML patients (BCR-ABL tyrosine-kinase oncprotein) and in the therapy of GIST patients (KIT receptor tyrosine-kinase).
exercise are called biosimilars (similar biological medicine product based on a comprehensive comparability exercise) and that have demonstrated non-inferior clinical efficacy and safety compared to the reference product and that have demonstrated non-inferiority with the reference product in a rational attempt to improve their clinical efficacy (Weise et al., 2011). Second-generation anti-CD20 antibodies with changed structure of the Fc portion of the antibody compared to reference anti-CD20 rituximab (e.g. GA-101) might serve as specific examples of second-generation biologicals (Alduaij et al., 2011; Niederfellner et al., 2011; Robak and Robak, 2011).

In conclusion, targeted therapy (with molecularly-targeted agents) and biological therapy (with biopharmaceuticals) represent two different categories of drugs. Only part of the emerging anti-cancer agents can be classified as biologicals, and their clinical usage as biological therapy. The same applies to molecularly-targeted agents. Biological agents may be molecularly-targeted (e.g. anti-CD20 rituximab), but may belong to non-targeted agents (e.g. interferon α). A wide range of molecularly-targeted drugs are not biologicals but belong to small-molecule chemical substances (i.e. to molecularly-targeted chemotherapy agents, e.g. imatinib, sunitinib, temsirolimus, enzastaurin, etc.) (Table 1). Despite the above-given clearly defined categories of drugs, there are many substances that are difficult to classify. We propose to categorize such anti-cancer drugs according to their physicochemical structure and hypothetical anti-tumour activity. Targeting the breast tumour oestrogen receptors (ER) with the non-steroidal anti-oestrogen tamoxifen might rather be classified as targeted (targeted) hormonal therapy (than targeted biological therapy). Retinoic acid (RA), which induces differentiation of acute promyelocytic leukaemia blasts, should...
be categorized as differentiating therapy. Histone-deacetylase inhibitors (vorinostat, panobinostat, romidepsin) or methyltransferase inhibitors (5-azacytidine, decitabine) should be classified as (non-targeted) epigenetic chemotherapy. The notion of immunomodulatory agents (IMIDs, i.e. thalidomide, lenalidomide, and pomalidomide) in our opinion represents a sufficient class designation for these low-molecular non-targeted drugs. Gene therapy by targeted gene replacement, alteration, or removal represents a separate treatment approach that is far beyond the scope and focus of this review. The same applies to pharmacogenomics, cancer vaccines, stem cell therapy, nanotechnology-based therapy, and perhaps other types of new treatment approaches not mentioned in this editorial.

The rational choice, dosage and timing of molecularly-targeted agent(s) based on pharmacogenomics and the presence of cancer-specific biomarkers in a particular patient with a particular malignancy in brief represents the concept of the so-called tailored/individualized/personalized therapy (Hamburg and Collins, 2010). In future, each patient with a malignant disease might theoretically receive a patient-specific cocktail of biological or chemical agents (targeted and/or non-targeted) at doses adjusted to the patient and to the cancer subtype. Personalized medicine thus struggles to fulfil the ultimate goal in the therapy of cancer that patients should be treated in a singular way, because each individual is unique and each malignancy different.

References


