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Review

From 'Targeted Therapy' to Targeted Therapy*

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Abstract. *In early 2000, the term 'targeted therapy' became popular and was used to indicate all types of tyrosine kinase inhibitors (TKI). However, the term targeted therapy had been used much earlier. Targeting tumor metabolism was already considered as targeted therapy, with methotrexate and 5-fluorouracil as the most successful examples. Hormone therapy is another successful type of targeted therapy. Imatinib was the first TKI for the fusion protein BCR–ABL and represented a breakthrough in the treatment of chronic myeloid leukemia. Many other TKIs have been introduced into the clinic, but most were less specific and had multiple targets, and therefore, by definition, not targeted. However, with the introduction of TKIs developed specifically against mutations in the active site of a TK, more truly targeted TKI have been approved, such as new anaplastic lymphoma kinase – echinoderm microtubule-associated protein-like 4 (ALK–EML4) inhibitors and the epidermal growth factor-T790M-targeted osimertinib. This article summarizes the content of the Burger-Kelland award lecture given by the Author in February 2019 during the 40th EORTC-PAMM Group meeting in Verona, Italy and reviews the development of various targeted agents.*

For targeted therapy, several definitions have been formulated. Sledge (1) summarized targeted therapy as follows: "A targeted therapy should attack a biologically important process (usually, though not necessarily, a single molecule), preferably one central to a hallmark of cancer. In its day, 5-fluorouracil (5-FU) was held up as targeted therapy, and appropriately so." Indeed, Heidelberger *et al*. (2) described 5-FU as targeted therapy, since this drug attacks metabolic pathways specific for a tumor.

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Definitions for targeted therapies varied in time and also within the same resource. The National Cancer Institute (NCI) glossary defined targeted therapy in 2012 as "a treatment that uses drugs to attack specific cancer cells", but in 2019 the definition included similar but more explanations: (i) Some block the action of certain enzymes, proteins or other molecules involved in growth of cancer cells; (ii) some types help the immune system to kill cancer cells; and (iii) most targeted drugs are small-molecule drugs or monoclonal antibodies (www.cancer.gov/dictionary). It should be realized that the term 'small molecule' is particularly vague, since the molecular weight of a drug such as 5-FU is 131, while that of gemcitabine is 266, methotrexate 454, doxorubicin 543 and imatinib 494. In the definition of the NCI, the differences between targeted therapies and conventional chemotherapy are summarized as follows: Targeted therapies act on specific molecular targets, were designed to interact with their target, and are often cytostatic (block proliferation), while standard conventional chemotherapy is designed to kill rapidly dividing tumor cells, may also affect dividing normal cells, and is cytotoxic (kill tumor cells). In reality, these differences are not black and white, since it has become clear that most TKI-based targeted therapies can affect normal cells leading to (sometimes lethal) toxicity. While conventional chemotherapies are often designed to inhibit one specific target, with the classic example of methotrexate, which only inhibits dihydrofolate reductase, many current new antifolates can be considered as targeted cytotoxic therapy (3), such as folate-receptor (FR)-targeted drugs that deliver their cytotoxic load specifically to tumor cells with high FR expression.

In this article, several types of targeted therapies are summarized (Table I), with some examples of conventional targeted drugs and TKIs in whose development the Author was involved.

Examples of Targeted (Chemo)Therapy

Targeted therapy in cancer cells is based on the inhibition of crucial enzymes/pathways in a cancer cell, for which two definitions can be formulated: Firstly, high activity of a pathway indicates that it may be essential "to drive the

Table I. *Types of targeted therapies that are available.*

1. Hormonal
2. Signal-transduction inhibitors (*e.g.* TKIs)
3. Gene-expression modulators
4. Apoptosis inducers
5. Angiogenesis inhibitors
6. Immunotherapies
7. Toxin-delivery molecules (targeted chemotherapy)
8. Gene therapy

TKIs: Tyrosine kinase inhibitors. Source: www.cancer.gov/dictionary.

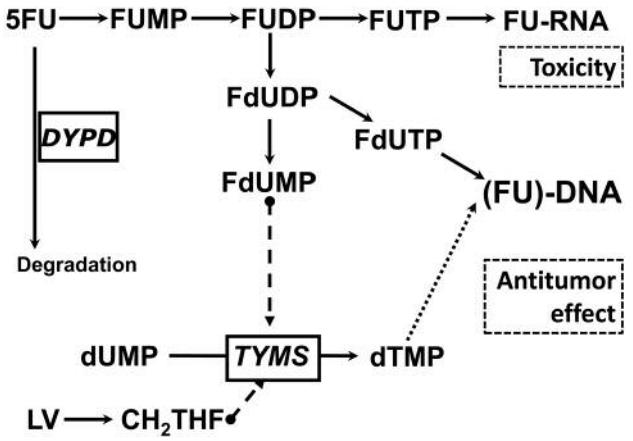

Figure 1. *Simplified metabolism and mechanism of action of 5-fluorouracil (5-FU), showing the activating pathways from 5-FU to FdUMP that inhibits thymidylate synthase (TYMS) by the formation of a ternary complex with TYMS and 5,10-methylene tetrahydrofolate (CH_2-THF), which is a metabolite of leucovorin (LV). TYMS inhibition leads to a depletion of dTMP and inhibition of DNA synthesis, which is responsible for the antitumor effect. The incorporation of FUTP into RNA is responsible for toxicity. Dihydropyrimidine dehydrogenase (DPYD) is responsible for 80% of the systemic degradation of 5-FU. Capecitabine (not shown) is activated by the sequential actions of carboxylesterase, cytidine deaminase (CDA) and uridine and thymidine phosphorylase to 5-FU.*

tumor" and its inhibition stops growth of (or sometimes kills) the cancer cell. Secondly, a low activity indicates that the activity may be rate-limiting and essential [according to Weber a 'key' enzyme] for growth, and therefore its inhibition may kill the cancer cell (4). In this case, the enzyme may be considered as the "engine". In contrast in normal cells:

The pathway may be absent or have lower activity
- Inhibition of an absent pathway is redundant
- When essential in normal cells, this will lead to toxicity.

A drug may act differently in normal cells.

Normal cells may have selective protection.

Normal cells may also depend on other pathways, so that the function of one pathway may be taken over by another.

Interestingly, for targeted chemotherapy, examples for both types can be found, such as thymidylate synthase (TYMS), for which it has been shown that a low expression in tumors is favorable for the action of 5-FU and other specific TYMS inhibitors, while in normal cells, 5-FU is predominantly incorporated into RNA, which is responsible for 5-FU-induced toxicity (5). Tumor cells with a high expression may be more sensitive to topoisomerase 1 inhibitors such as irinotecan. For TKIs, it has been shown that a high expression (or activating mutations such as for epidermal growth factor (EGFR) is favorable for EGFR-TKIs such as erlotinib and gefitinib (6). Interestingly for another group of kinase enzymes, the so-called nucleoside kinases (including ribonucleoside, deoxynucleoside and nucleotide kinases), a high activity is favorable for their activity such as that for deoxycytidine kinase (dCK) (7), which is associated with a better antitumor effect of gemcitabine, a deoxynucleoside analog activated by dCK. Chemotherapy can be made targeted by the design of proper combinations, which in an ideal situation will increase the effect against cancer cells, but protect normal cells.

A classical example of a targeted cytotoxic drug is 5-FU (Figure 1). 5-FU is a prodrug, which has to be activated to its nucleotide FUMP, which is mediated by either direct phosphoribosylation or by the coupled uridine phosphorylase/uridine kinase reaction (8). *Via* three steps, the active metabolite FdUMP is formed, which is a potent suicide inhibitor of TYMS by the formation of a ternary complex between FdUMP, TYMS and 5,10-methylene tetrahydrofolate (CH_2THF). Leucovorin is given to increase the concentration of CH_2THF and stabilize the ternary complex. In contrast to what has been postulated repeatedly in literature [reviewed in (5)], the coupled enzyme reaction thymidine phosphorylase/thymidine kinase is unlikely to be involved in the direct formation of FdUMP from 5-FU, simply because the essential co-substrate 2-deoxy-ribose-1-phosphate is not present in tumor cells (9). However, both thymidine phosphorylase and uridine phosphorylase are responsible for the activation of the 5-FU prodrug capecitabine to 5-FU. Several groups, including ourselves (10), have shown that a low expression of TYMS and high TYMS inhibition is associated with a higher response rate and/or a longer survival in patients with advanced colorectal cancer [reviewed in (11)]. In contrast, incorporation of 5-FU into RNA was not associated with the antitumor effect of 5-FU (12), but with both hematological and gastrointestinal toxicity of 5-FU. Normal cells can be protected from such toxicity by post treatment with a high dose of uridine (13). This led to the development of triacetyl uridine (https://www.wellstattherapeutics.com/therapeutics/VPR.pdf), which is registered

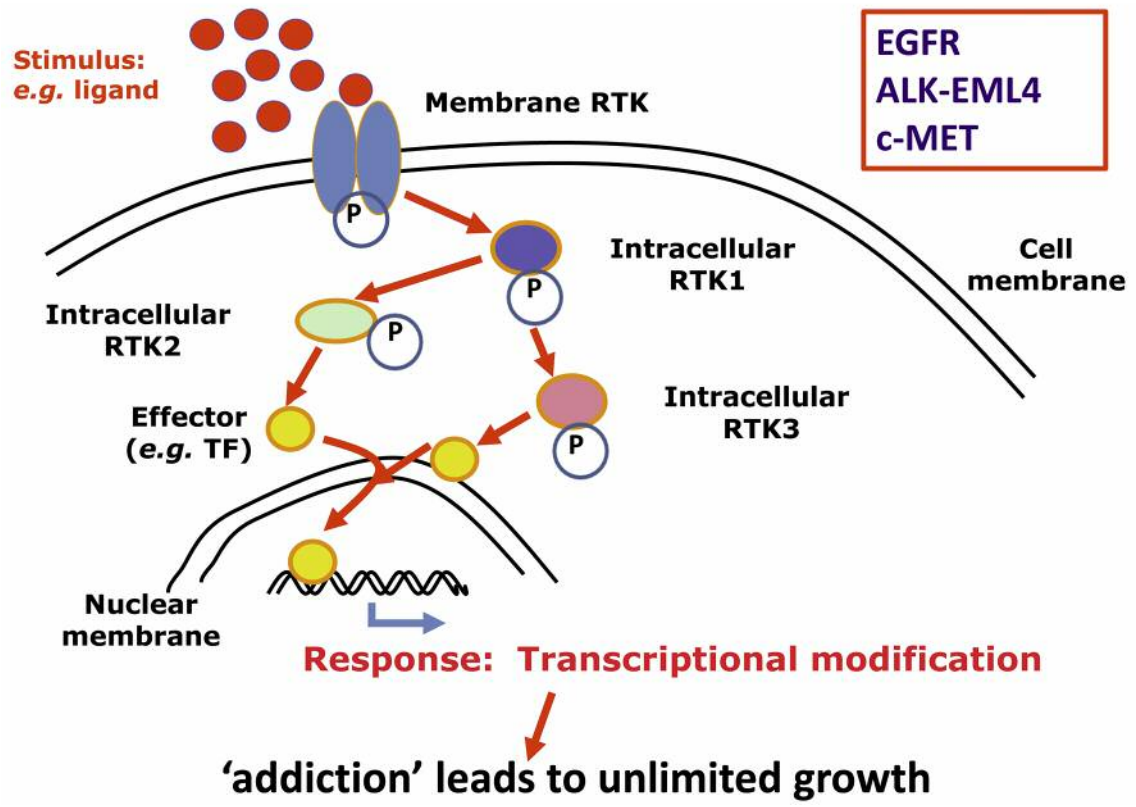

Figure 2. *Simplified scheme for the mechanism of action of receptor tyrosine kinases (RTK), e.g. epidermal growth factor (EGFR), ALK–EML4 and c-MET. Membrane-bound RTK is activated by a ligand, leading to autophosphorylation of the intracellular domain, which can lead to a cascade of phosphorylation of intracellular RTKs, which may act in parallel to activate an effector, which is usually a transcription factor (TF). Tumor cells are often addicted to one of these RTKs or pathways.*

for rescue treatment of 5-FU-induced toxicity, including patients with a deficiency of dihydropyrimidine dehydrogenase (DPYD) who may suffer from lethal 5-FU toxicity when receiving a full dose.

The association of TYMS expression with the antitumor activity of 5-FU led to a prospective study in which patients were selected for 5-FU–leucovorin precision therapy when they had a low expression of both TYMS and DPYD in the tumor (14). Patients with low TYMS and DPYD expression selected for 5-FU–leucovorin had a prolonged median overall survival of 23.6 months compared to a historical median overall survival of 11 months, or 13.2 months in the high TYMS and/or DPYD group treated with irinotecan and oxaliplatin. Unfortunately, this study was not followed up since the 5-FU leucovorin combination was replaced by combinations with either oxaliplatin (FOLFOX and CAPOX) or irinotecan (FOLFIRI and CAPIRI). For another drug targeting TYMS, the antifolate pemetrexed, it was demonstrated that a low TYMS expression was associated with a better antitumor effect in patients with mesothelioma (15).

These few examples show that rationally designed conventional chemotherapy can be targeted when proper patients are selected, focusing on the expression of the relevant targets in the tumor. More recently, it was finally demonstrated for 5-FU that patients should be characterized for the expression/activity of the systemic degradation enzyme DPYD (16), a concept already proposed by us in the 1990s (17). Similarly, it is proposed to adapt the gemcitabine dose based on the activity of the degradation enzyme cytidine deaminase (CDA) (18).

Expression of Tyrosine Kinases in Tumors

The general mechanism of action of receptor tyrosine kinases (RTK) is depicted in Figure 2 (19). An RTK needs to be activated by a specific ligand, which binds to it on the outside of the cell membrane, usually leading to auto phosphorylation of the intracellular TK domain, which initiates a cascade of activation of RTKs, varying from one to multiple TKs, often of parallel pathways. Ultimately, this

leads to activation of an effector, usually a transcription factor, which ultimately makes TKIs also DNA targeted drugs. When one of the RTK is activated in a tumor cell, this often leads to an "addiction" of the tumor cell to this pathway. This addiction makes these tumor cells attractive targets for RTK targeted drugs, such as erlotinib and gefitinib for amplified or mutated (activating mutations) EGFR-TK (6), crizotinib for amplified or mutated c-MET receptor (20).

The first- and second-generation EGFR-targeted drugs are active against patients with NSCLC with activating mutations (the in-frame deletion in exon 19, delE746-A750, and the missense point mutation L858R), without mutations in intracellular pathways such as *KRAS*, *PTEN* and *AKT*. Almost all patients with NSCLC develop resistance within 1 year of erlotinib or gefitinib therapy (21), often due to a mutation in the active kinase domain, the T790M mutation. Several specific inhibitors were developed based on the 3-D structure of the mutated TK domain, including the third-generation agents rociletinib and osimertinib, the latter was initially registered for second-line therapy of patients with T790M mutation, but osimertinib is now indicated for first-line treatment. Because of its specific activity against the mutated RTK domain, osimertinib can be considered as an example of a new truly targeted TKI.

However, treatment with third-generation TKIs still leads to resistance (21), which is either in the kinase target itself, often a C797 mutation, or by activation of alternative pathways such as the NF-κB pathway (22) or *c-MET*. Specific C797-targeted inhibitors are currently being developed. The presence of c-MET amplification offers the possibility to combine erlotinib or osimertinib with the c-MET inhibitor crizotinib (23).

Another example of successful development of a truly targeted TKI is crizotinib, and analogs derived therefrom. Crizotinib attacks the c-MET receptor (20), which is activated by hepatocyte growth factor, and stimulates proliferation, transformation, migration and tubulogenesis. This makes c-MET an attractive target, especially in tumors with an amplified or mutated c-MET receptor. In selected pancreatic ductal adenoma carcinoma, overexpression of c-MET was found (24). Orthotopic patient-derived xenografts were sensitive to crizotinib and synergistic with gemcitabine. increasing the accumulation of the active gemcitabine metabolite dFdCTP and the accumulation of crizotinib in the tumor. This is an excellent example of a synergistic interaction between a TKI and the conventional chemotherapeutic drug gemcitabine. Therefore a prospective clinical study on pancreatic ductal adenoma carcinoma is proposed in which patients should be selected for their c-MET expression, similarly to the CREATE study (25), in which patients with six different rare malignancies (including papillary renal cell cancer, sarcoma, rhabdomyosarcoma) were selected for their *c-MET* expression (wild-type *versus* amplified/mutated) and when positive received treatment with crizotinib. Patients with papillary renal cell cancer with amplified/mutated *c-MET* had a significantly longer progression-free survival (median >2.5 years) compared to patients in which *c-MET* was not amplified. This study shows that TKIs can be targeted and be used for precision therapy.

Conclusion

It can be concluded that (i) the definition of targeted therapy has changed repeatedly in the past century; (ii) conventional chemotherapy can be targeted; (iii) in the past decade, TKIs were inappropriately considered as being targeted. Moreover, almost all TKIs are 'dirty' multitargeted drugs; and are often similarly or even more toxic than 'cytotoxic' therapy. However, TKIs are a new form of cytostatic chemotherapy and are a very valuable addition to current cancer therapy.

The past decade has also shown that development of TKIs is most efficient when they are designed very specifically to act against an active site, and can bypass resistance when they are designed to target a modified amino acid. Proper absorption, distribution, metabolism, excretion, toxicology and drug pharmacokinetic properties are equally important for development of new TKI as for any other conventional drug (19).

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Conflicts of Interest

None to be reported for this article.

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