REVIEW ARTICLE

THERAPEUTIC POTENTIAL OF ANGIOGENESIS INHIBITOR MIXTURES IN CANCER TREATMENT

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ABSTRACT

It is generally understood that improvement of cancer therapies is achieved by the combination of drugs. This approach will improve efficacy, while toxicity and the risk for drug resistance may be decreased. In this review, the efforts on combining drugs for the treatment of two tumor types is highlighted, followed by a description of the challenges that are faced in designing optimal combination therapies.

INTRODUCTION

Over the recent years significant advances have been made in the treatment of advanced stage tumors. Cytotoxic chemotherapy regimens have improved and overall survival in patients has increased. But most therapies are associated with severe systemic toxicities [1,2]. Angiogenesis inhibitors such as bevacizumab/Avastin®, panitumumab/Vectibix®, sunitinib/Sutent®, sorafenib/Nexavar® and erlotinib/Tarceva® are used in clinical practice in many tumor types as single drugs or in combination with chemotherapeutics [1,3-6]. These agents target and perturb critical cellular signaling pathways that regulate tumor angiogenesis. The efficacy of such combinations strongly depends on many factors, among which are the treatment schedule, the genetic background of the patient and the choice for the combination of drugs [5,7].

To date, the clinical benefit of anti-angiogenic agents has been variable, depending on the tumor type, and in most cases only a limited survival increase was noted. Major causes for this limited success are the occurrence of side effects and the induction of resistance. It is generally believed that a major progress in cancer treatment can be achieved by finding optimal combination therapy strategies. This made the search for optimal drug combinations among the fastest growing topic in the field of cancer research. However, concerns regarding toxicity and drug resistance still constitute barriers to be overcome [8].

We have recently published an overview on current efforts of combining anti-angiogenesis drugs with other treatment modalities such as such as chemo-, radio-, immuno- or photodynamic therapy (PDT) and several advances in these efforts were reported [9]. This review explores the emerging issue on the therapeutic potential of optimized angiostatic drug combinations by analyzing clinical data for two tumor types, hepatocellular carcinoma (HCC) and renal cell carcinoma (RCC).

ANTI-ANGIOGENIC DRUG COMBINATIONS BASED ON CLINICAL PRACTICE. THE LESSONS WE LEARNED

The selection of drugs for use in combination therapies in the clinic is often based on the previous success of individual drugs as monotherapies [10]. This problem was well illustrated by the following quote of Robert Weinberg [11]: *"Traditionally, new drugs have been evaluated as single agents during pre-clinical development and Phase I clinical trials. This practice contrasts with the growing belief of cancer researchers that most monotherapies are unlikely to yield curative treatments and that, with rare exceptions, truly successful clinical outcomes will depend on the use of combinations of anti-cancer drugs".*

Moreover, in most cases new molecules tested in the first-line setting need to be combined with the standard of care compound to demonstrate superiority. It is clear that such an approach seems to rely on trial and error and does not take into consideration the drug-drug interactions and their influence to the tumor microenvironment. In the following section, we analyze the clinical results of targeted treatments in two examples of advanced malignancies.

Hepatocellular carcinoma (HCC) is a highly vascularized tumor type that is treated with anti-angiogenic agents. The only systemic drug approved for treatment of unresectable HCC is sorafenib [12]. This treatment modality causes, nevertheless, major toxicities, such as hand-foot syndrome, skin reactions, diarrhea and fatigue [13], while it induces both primary and acquired resistance [14]. The efficacy of sorafenib is probably due to a good balance between targeting cancer cells and their microenvironment, as a result of blocking multiple kinases (including VEGF, PDGF, C-KIT, and B-RAF). However, with this treatment the life expectancy of these patients is still only around 12 months, indicating a clear need for better treatment strategies. The reason for failure could be the large heterogeneity of both patients and tumors, as well as the lack of understanding of critical drivers of tumor progression or dissemination.

Numerous clinical trials have been conducted to evaluate a large number of molecularly targeted drugs for treating HCC in the first-line (brivanib, sunitinib, erlotinib, linifanib or tivantinib) or second-line (brivanib, everolimus), but most drugs exhibited less efficacy and/or higher toxicity as compared to sorafenib (see Sun et al. for a comprehensive review [15]. Bevacizumab showed promise as an effective and tolerable treatment for advanced HCC. The reported efficacy of bevacizumab appeared to compare favorably with that of sorafenib [16].

Despite the fact that anti-endoglin therapy with TRC105 in phase II studies in patients with advanced HCC - post-sorafenib - brought evidence of clinical activity, it was not promoted to the second stage of investigation [17]. Tivantinib, a selective oral inhibitor of MET, has shown promising antitumor activity in HCC as monotherapy. In a randomized phase II study, time to progression was longer in patients treated with tivantinib as compared to placebo group. This result was unfortunately accompanied with grade 3, or worse, neutropenia and anaemia [18]. Everolimus, the mTOR inhibitor, was used in a randomized phase III trial (EVOLVE-1) in the second-line treatment in advanced HCC patients who experienced failure of sorafenib treatment. No significant overall survival benefit was observed in comparison with the placebo group [19]. These examples of treatment failure in the management of HCC add further insight to the need to change the current mindset of trial design. Different treatment roads should be sought and urgently translated into the design of early clinical trials in order to maximize the chances of positive clinical outcomes.

There are only a few clinical trials with an arm of evaluating the simultaneous administration of two anti-angiogenic agents in HCC. The combination of bevacizumab and erlotinib was reported to have high clinical activity for HCC by a phase II study conducted in the USA, however, it showed modest activity in an Asian cohort [20]. This might very well be that genetic/racial backgrounds play a significant role in treatment design and outcome.

Refametinib, an allosteric MEK inhibitor, has been reported to possess antitumor activity in combination with sorafenib in preclinical models [21]. In a phase II study evaluating efficacy and safety of this combination HCC it appeared to be clinically active, although the high incidence of dose modifications may have compromised the efficacy results. Interestingly, the subgroup of patients with RAS mutations seemed to respond the most from this treatment [22].

Another pivotal example of the clinical use of anti-angiogenic therapy is the treatment of advanced renal cell carcinoma (RCC). The kidney is a heavily vascularized organ and it is probably for this reason that RCC is among the most vascularized malignancies [23]. Anti-angiogenic agents demonstrated significantly greater antitumor effects in RCC compared with the previous standard first-line therapy with interferon-a (IFNa). Also for RCC, as was the case for HCC, the limited activity of single drug therapies is mainly due to disease and patient heterogeneity [24], toxicity [25-28] and possible enhancement of metastasis [29] and drug resistance [30-32]. Many preclinical and clinical efforts have focused on combining existing agents or sequencing of them to maximize their impact on clinical outcomes. A comprehensive systematic review of sequencing and combinations of systemic therapy in metastatic renal cancer has recently been presented by Albiges et al. [33]. Often, the choice of therapy is based on a patient and physician decision, which is based on comorbidities, toxicity profiles and costs. The treatment selection from the multiple choices that exist now for the first- and second-line treatment of patients with mRCC became substantially complex by the possibility of combining drugs and by their sequencing options [6]. When applied in combination, next to – or instead of - enhanced efficacy, substantial toxicity has been a recurrent observation in these studies [33,34], even when designed to target complementary pathways [35]. Large studies such as the RECORD, INTORACT, CALGB and TORAVA studies [36-39] investigated first-line combination regimens and did not prove, in most cases, superior outcome over single agents.

The activity of sorafenib, temsirolimus, and bevacizumab administered in doublet combinations (e.g. the BEST trial) did not significantly improve median progression-free survival in comparison with bevacizumab monotherapy [40]. The list of available clinical trials is very long [41] and the current (2015) European Association of Urology (EAU) guidelines for treatment of mRCC recommend nivolumab (a programmed death 1 (PD-1) checkpoint inhibitor) [42] and cabozantinib (targets VEGFR, as well as MET and AXL) [43] used in sequence over the previous standard of care (everolimus) in patients who have failed one or more lines of VEGF targeted therapy [40].

BACK TO THE DRAWING BOARD: HOW TO DESIGN MORE EFFECTIVE MULTIDRUG TREATMENTS?

The information given in the two above mentioned tumor types, shows that the trial and error approach in combining previously tested promising agents is by far not a systematic way of finding the best combination treatment. Although it is logical to assume that drugs with a positive anti-tumor effect will synergize with other drugs that have shown promise. However, drugs may also synergize or have synergies without proof of efficacy as a monotherapy. Not to talk yet about which drug doses should be used. There is simply not enough time, neither there are enough cancer patients, to search systematically for optimal combination treatments.

Nevertheless, the above clearly demonstrates that one of the keys for improvement of cancer therapy in general, is the combination of existing drugs. Three main challenges exist in the effort of optimizing multi-drug combinations: (i) the complex nature of a biological system, which makes it virtually impossible to predict optimal drug combinations based on empirical information, (ii) the large number of possible drug combinations that exist when multiple drugs are considered at multiple concentrations [44], and (iii) optimization of drug administration in a pre-designed sequence in order to minimize the impact of drug resistance or escape mechanisms.

In clinical settings, a maximum tolerated dose (MTD) approach was used to define tolerable drug concentrations, but several of the trials reported that the MTD was not well tolerated and lower doses may have been more appropriate. Moreover, little consistency with the establishment of MTD based on dose limiting toxicities (DLTs) was observed as several of the trials measured DLTs. The sequential use of presently available molecularly targeted agents has become a standard in everyday clinical practice, especially in RCC. The available data for novel therapies in mRCC suggest they can be used sequentially across multiple lines of treatment to extend survival (see the current EAU guidelines for mRCC in a chapter above). Up to date, the results from both combination and sequencing clinical trials suggest that these approaches can be feasible, but prospective data on expected benefit are still missing, and new treatment tailoring methods are needed.

Many groups have investigated methods to optimize drug combinations, including techniques to model cancer progression and predict cell responses [45,46], systematic searches [47], as well as technologies using various deterministic and stochastic search algorithms [48-50] or sequential decoding algorithms [51]. In previous research, we have used the feedback system control (FSC) technique with a population-based stochastic search algorithm to navigate in the experimental parametric space of nine angiostatic drugs applied at four concentrations. For many biological applications, the FSC scheme is ideal, because it requires no knowledge of the mechanisms involved in determining the cellular response to a given drug stimulus or input. It only requires an output value that describes the overall cellular activity in response to a drug combination. This output is fed into the closed-loop feedback system, in order for the search algorithm to determine the next iteration of drug combinations to be tested on the cell system 44. FSC has already been successfully used in various complex biological systems, including the inhibition of viral infection [52], maintenance of human embryonic stem cells [53], the differentiation of mesenchymal stem cells [54] or nanodiamond-modified drugs [55].

By an iterative approach of in vitro testing for endothelial cell (EC) viability and applying a second-order linear regression analysis, we used this procedure of elimination of less effective drugs and identify the optimal synergistic, low-dose drug combination. Drug doses were reduced by 5- to 11-fold, as compared to optimal single drug concentrations. The three remaining drugs were found to target distinct and complementary signaling pathways. One might expect that the differences in pharmacokinetics and pharmacodynamics between the components of the drug mixture may interfere with the in vivo treatment outcome. However, the flat response surfaces, which are the graphic illustration of drug combination-drug efficiency landscapes, allow for a much simplified translation from the optimal in vitro combination to in vivo application (i.e. moderate changes in drug ratios do not significantly change the output). Thus, successful "ratiometric" translation of the synergistic anti-cancer activity of this drug combination was proven in two preclinical in vivo tumor models, i.e. human ovarian carcinoma and colorectal carcinoma.

Indeed, in two tumor models we have observed an effective tumor growth inhibition clearly driven via anti-angiogenic mechanism [56].

Summarizing, the three major advantages of the proposed personalized technology (i.e. rapid development of a patient-specific optimal drug mixture, that is adjusted during the course of treatment while the cancer progresses) are: (i) the possibility to reduce side effects, (ii) the decreased probability of developing drug resistance and (iii) the relative speed of the FSC-based optimization process which would facilitate its clinical application. We believe that FSC can also be the gateway to a reliable method for the design of personalized optimal combination treatments.

CONCLUSIONS AND FUTURE DIRECTIONS

Clinical trials are currently evaluating whether therapeutic benefit can be improved by combining agents that block multiple levels of the same or different pathways, thus providing additive or even synergistic effects. It remains to be determined if systematic

computer guided identification of optimized reduced-dose drug combinations would provide maximum efficacy and tolerability benefits in patients. The attractiveness of finding optimal drug combinations, using targeted compounds, for the inhibition of angiogenesis, is the broad application opportunities. Treatment of many cancer types may be improved, but also application in other angiogenic diseases, such as atherosclerosis [57,58], rheumatoid arthritis [59] and endometriosis [60, 61] is feasible.

ABBREVIATIONS

EC: endothelial cell; EAU: European Association of Urology; FSC: feedback system control; HCC: hepatocellular carcinoma; IFNa: interferon-a; MTD: maximum tolerated dose; PDT: photodynamic therapy; PD-1: programmed death 1; RCC: renal cell carcinoma.

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