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APPLICATION OF MOLECULAR TARGETED THERAPY IN PATIENTS WITH ADVANCED COLORECTAL CARCINOMA

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Summary

The introduction of targeted therapies has made substantial progress in advanced colorectal cancer treatment. Compared to chemotherapy, which is unselective – cytotoxic for both healthy and malignant cells and thus causes multiple adverse events, the targeted therapy is directed upon specific tumor cell markers. This leads to lower toxicity and improves therapy results. There are several groups of agents used in targeted therapy and two are in clinical use: monoclonal antibodies and small molecules – inhibitors of tyrosine kynase. Monoclonal antibody therapy is highly tumor specific with low toxicity. Two main functions of antibodies include recognizing and binding of antigens, and subsequently provoking immunological response of the patient. Small molecules act as inhibitors of tyrozine kynase intracellular domain, preventing phosphorilation and intracellular signal transduction. The tagreted therapy of colorectal cancer is directed upon EGFR (epidermal growth factor receptor) and its intracellular signal transduction as well as neoangiogenesis.

KEY WORDS: *advanced colorectal cancer, epidermal growth factor receptor inhibitors, vascular endothelial growth factor inhibitors*

PRIMJENA MOLEKULSKIH CILJANIH LIJEKOVA U BOLESNIKA S PROŠIRENIM RAKOM DEBELOG CRIJEVA

Sažetak

Uvođenjem ciljanih terapija postignut je znatan napredak u liječenju uznapredovalog raka debelog crijeva. U usporedbi s kemoterapijom, koja je neselektivna – citotoksična i za zdrave i zloćudne stanice te ima višestruke štetne učinke, ciljana terapija usmjerena je prema specifičnim obilježjima tumorskih stanica. Time se postiže manja toksičnost i bolji rezultati liječenja. U ciljanoj terapiji primjenjuje se nekoliko skupina lijekova, a dvije su u kliničkoj uporabi: monoklonska protutijela i male molekule – inhibitori tirozin kinaze. Monoklonska protuutijela djeluju vrlo specifično na tumor, a toksičnost im je mala. Dvije glavne funkcije protutijela jesu prepoznavanje i vezanje antigena, a time i poticanje imunološkog odgovora u bolesnika. Male molekule djeluju kao inhibitori tirozin kinaze u unutarstaničnoj domeni te priječi fosforilaciju i unutarstanični prijenos signala. Ciljana terapija raka debeloga crijeva usmjerena je na receptor epidermalnog čimbenika rasta i unutarstanični prijenos signala te neoangiogenezu.

KLJUČNE RIJEČI: *proširenи rak debelog crijeva, inhibitori receptora epidermalnih čimbenika rasta, inhibitori čimbenika rasta krvožilnog endotela*

INTRODUCTION

A wide range of signal transduction pathways have been identified as critical for the growth and proliferation of solid tumors. There is also growing evidence that many of these signaling pathways are involved with other key cellular events including DNA repair, cell survival signals, invasion/metastasis and angiogenesis. Moreover, many of these signaling pathways

may play a key role in mediating sensitivity to chemotherapy and/or radiation therapy. Progress in tumor biology research has improved the understanding of the role of growth factors, cell surface receptors, and their second messengers in the tumor development and progression, thus the modulation of these factors is an important anticancer strategy (1). Several approaches have been developed to inhibit the signal transduction pathways, and they include: 1) agents directed to interfere with the self-sufficiency in growth signals, such as epidermal growth factor receptor (EGFR) inhibitors; 2) agents directed to inhibit the angiogenesis process; 3) agents directed to interfere with the limitless replicative potential, such as cell cycle inhibitors; 4) agents directed to promote apoptosis, such as proteasome inhibitors; and 5) agents directed to inhibit the tissue invasion and the metastatic processes, such as matrix metalloproteinases inhibitors (2).

Despite an improvement appreciation of the factors involved in the malignant transformation and progression of colorectal cancer, this type of tumor remains one of the most common malignancy and the second leading cause of cancer mortality. Colorectal cancer belongs to the tumor model with overexpressed signal transduction pathways that are potential targets of a number of new drugs that are currently being developed. Two main categories of compounds have been developed: agents that target the extracellular domain of the receptor and those that target the intracellular tyrosine kinase (TK) domain. Approaches to target the extracellular, ligand-binding domain include monoclonal antibodies (mAbs) that directly interfere with receptor signaling or serve as delivery systems for radionucleotides, toxins, or prodrugs. Small molecule tyrosine kinase inhibitors (TKIs) can interfere with catalytic activity of the cytoplasmatic domain or alter downstream signal propagation. This two strategies have been tested in the clinical setting, and there is evidence that both monoclonal antibodies and TKIs have clinical activity (3-5).

EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITORS

The epidermal growth factor receptor (EGFR), also known as HER1 or ErbB1, is a 170-kD glycoprotein that consists of an extracellular ligand-binding domain, a hydrophobic transmembrane region, and an intracellular TK domain. EGFR belongs to the family ErbB, of wich four receptors have been characterized: HER1, HER2 (neu or erbB-2), HER3 (erbB-3) and HER4 (erbB-4) (6). Ligands for EGFR include EGF, transforming growth factor-alfa (TGF-α), amphiregulin, heparin-binding EGF, betacellulin and epiregulin. EGRF exists as a monomer in the cell membrane. Once a ligand binds to the extracellular domain of EGFR, receptor dimerization occurs. Dimerization is followed by TK activation and autophosphorylation on tirosine residues, key steps to the initiation of downstream physiologic and pathogenic events. As a consequence, signaling pathways such as Ras-Raf-MAP kinases, phosphatidylinositol 3-kinase, Akt, Jak/Stat kinases, and protein kinase C are activated and gene transcription is regulated (7).

CETUXIMAB

Cetuximab (Erbitux) is a chimeric immunoglobulin IgG1 monoclonal antibody that was initially developed from murine antibody M225 developed by Mendelsohn and colleagues.[4] It functions as a competitive antagonist that recognizes the extracellular domain of the EGFR and thereby competes with its ligands for receptor occupation. Upon binding, cetuximab induces receptor dimerization and endocytosis, resulting in EGFR downregulation from the cell surface, cell-cycle arrest, and cell death. Phase II trials have shown activity of cetuximab as a single agent and in combination with both irinotecan and oxaliplatin based chemotherapy in previously treated patients with metastatic colorectal cancer. A large, phase II randomized, multicentric study (the «BOND» trial) conducted by Cunningham et al. (8) showed impressive activity alone or in combination with irinotecan in patients with irinotecan-refractory disease. The overall response rate for 218 patients who received combination irinotecan plus cetuximab was 22.9% (p=.007), while 111 patients who received cetuximab alone demonstrated a 10.8% response rate. The median time to progression was 4.1 months for the combination and 1.5 months for the cetuximab monotherapy group. Several

clinical studies have examined whether cetuximab improves the efficacy of the first-line chemotherapy regimens FOLFOX (5-FU/leucovorin/oxaliplatine) or FOLFIRI (5-FU/leucovorin/irinotecan) in untreated patients with advanced colorectal cancer. An international phase II study (ACROBAT) has shown that cetuximab improves treatment outcomes in combination with FOLFOX4 in the first-line therapy of patients with EGFR-overexpressing stage IV disease. The overall response rate for patients who received combination therapy was 81%, while patients who received FOLFOX4 alone demonstrated a 45% response rate. An additional seven patients experienced stable disease, leading to an overall disease control rate of 98% (9). Clinical data also show the addition of cetuximab to FOLFIRI to be safe and potentially efficient first-line treatment for advanced colorectal cancer. Results of a recent phase II trial also show the activity of cetuximab plus bevacizumab with or without irinotecan in patients with advanved colorectal cancer who failed irinotecan, oxaliplatin and fluoropyrimidines (10).

Because EGFR expression may be a prognostic indicator and may increase the likelihood of capturing antitumor activity, there has been no clear association between EGFR expression and response to EGFR-targeted therapy in colorectal cancer. Dermatologic toxicities are the most frequently reported side effects associated with the currently available anti-EGFR therapies. In phase II clinical trials of cetuximab, erlotinib and gefitinib, the development of a moderate rash (>grade 2) has been associated with improved median survival. Infusion reactions are unique to mAbs, whereas diarrhea and interstitial lung disease appear to be predominantly associated with TKIs. Hypomagnesemia has recently been recognized as a relevant side effect of cetuximab therapy (7).

Other anti-EGFR mAbs that have similar mechanism of action to cetuximab are in clinical development. Matuzumab (EMD7200) is a humanized IgG1 mAbs with high affinity currently in phase I and II development. This mAb has a prolonged half-life that may allow for a less frequent administration schedule. In an ongoing trial, pharmacokinetic, pharmacodynamic and efficacy data indicate that a more convenient schedule with matuzumab every 2 or every 3 weeks is feasible. Panitumumab (ABX-EGF) is a fully human IgG2 mAb with high affinity for the EGFR. In a phase I/II study of panitumumab in patients with advanced refractory colorectal cancer, this mAb showed a similar efficacy to cetuximab in this population (7, 11).

There are a large number of TKIs directed to the EGFR family in clinical development.

GEFITINIB

Preclinical studies suggested that gefitinib (ZD1839, Iressa) may have significant single-agent activity in colorectal cancer. Gefitinib is an EGFR tyrosine-specific quinazoline with excellent oral bioavailablility that has demonstrated cytostatic antitumor activity with chronic administration in preclinical evaluations. Similar to the other EGFR-targeted agents, antitumor activity was enhanced when coadministered with cytotoxic therapies, including those with atitumor activity in colorectal cancer. A multicenter phase II study by the Eastern Cooperative Oncology Group (ECOG 6200) of 115 patients with metastatic colorectal cancer failed to demonstrate any single-agent activity when judged by radiographic response rates. In patients with metastatic colorectal cancer gefitinib has been investigated in combinations with 5-FU and leucovorin, oxaliplatin, as well as capecitabine (12). Some antitumor activity has been observed with these regimens, but the results are preliminary.

ERLOTINIB

Erlotinib (OSI-774, Tarceva) is highly specific and reversible, ATP-competitive, orally bioavailable, quinazoline inhibitor of the EGFR tyrosine kinase. Minor responses in patients with colorectal cancer were observed in the phase I studies and disease-specific phase II studies have been initiated in colorectal cancer. Preliminary data from a phase II study in 30 patients with metastatic colorectal cancer indicate that the best response to therapy is stable disease (stable disease was observed in 32% of patients, but no responses have been seen) (13).

VASCULAR ENDOTHELIAL GROWTH FACTOR INHIBITORS

Angiogenesis, the process of new blood vessel formation, is considered critical for the growth of tumors. Angiogenesis inhibition represents a new paradigm in the treatment of cancer. Unlike traditional chemotherapy, angiogenesis inhibitors specifically target the formation of new blood vessels. The formation of vascular network in the tumor growth involves different complex pathways including angiogenic factors, membrane receptors and signaling transduction cascades, leading at the end to vessel formation. There is preclinical evidence showing cross-talk between angiogenic and tumor growth signaling pathways. One recent study suggest that the activation of the EGFR-pathway contributes to angiogenesis (14). The vascular endothelial growth factor (VEGF) is the most potent and specific angiogenic factor. The VEGF family consists of six glycoproteins, including VEGF-A,-B,-C,-D,-E and placental growth factor. VEGF expression is regulated by numerous factors, including other angiogenic factors and cytokines, hypoxia, and many tumor oncogenes. In general, VEGF family members mediate their effects by binding to one or more of the VEGF receptors and/or coreceptors, leading to dimerization, with resultant phosphorylation and activation of the receptor's intracellular tyrosine kinase domain. There are three members of the VEGF receptor family, including VEGFR-1,-2 and -3. VEGF's downstream signaling is also complex, with activation of the Ras-Raf-ERK, Src-FAK, AKT-mTOR, and small G-protein-eNOS (epithelial nitric oxide synthase) pathways (15, 16).

BEVACIZUMAB

Bevacizumab (Avastin) is a 149-kD recombinant humanized monoclonal IgG1 antibody that consists of appromaximately 93% human framework domains and 7% murine-derived bindings domains (17). The antibody selectively bloks binding of the ligand VEGF with its receptors, thereby preventing VEGF activation of VEGFR-1 and VEGFR-2 receptors. Initial phase I studies demonstrated a convenient 17- to 21-day half-life and no significant toxicities at doses that reduced free plasma VEGF to undetectable levels. Two completed phase III trials support the use of bevacizumab with combination chemotherapy. The first compared IFL (irinotecan/5-FU/leucovorin) with and without bevacizumab (18). The third arm was discontinued once the safety of IFL plus bevacizumab was established. The addition of bevacizumab 5 mg/kg biweekly significantly improved the primary outcome of median survival from 15.6 months with IFL alone to 20.3 months with IFL/bevacizumab (p= .0003). Bevacizumab also significantly increased response rate from 34.8% to 44.8% and prolonged time to progression from 6.2 months to 10.6 months. An Eastern Cooperative Oncology Group phase III study (E3200) accrued 828 patients who had failed previous therapy with both irinotecan and fluoropyrimidine to one of three arms: FOLFOX with and without bevacizumab, and bevacizumab alone (19). Five hundred seventy-nine patients were enrolled on one of the FOLFOX-containing regimens. The addition of bevacizumab biweekly significantly prolonged overall survival from 10.7 months to 12.5 months.

More recently, the addition of bevacizumab to oxaliplatin-based combinations in the first-line treatment of metastatic colorectal cancer has been evaluated (20). The TREE-2 trial was developed to compare bevacizumab in combination with 3 different oxaliplatin plus fluoropyrimidine regimens. Patients were randomly assigned to receive either FOLFOX-B (with infusional 5-FU), bFOL-B (with bolus 5-FU), or CapOx-B (with oral capecitabine). The incidence of grade ¾ neutropenis was 35% in patients receiving FOLFOX-B, 13% in those receiving bFOL-B, and 4% in those receiving CapOx-B (p< .001). The preliminary results of TREE-2 suggest that the addition of bavacizumab to the 3 oxaliplatin/fluoropyrimidine combinations did not produce significant additive toxicity compared with those from the TREE-1 trial. The overall best response rate for the TREE-2 trial was 62% in the FOLFOX-B arm, 43% in the bFOL-B arm, and 57% in the CapOx-B arm. Based on these data, the authors concluded that FOLFOX-B appeared to have the best balance of response and toxicity. Similar preliminary results are reported from a phase II trial in which bevacizumab was added to capecitabine and oxaliplatin (XELOX). The combination re-

sulted in promising overall response rate of 57% and time to progression of 11.9 months.

Adding bevacizumab to both first and second line chemotherapy improves response and overall survival, but not without toxicity. Phase I and II trials recorded an increased risk of bleeding, clotting, hypertension, and bowel perforation in patients receiving the angiogenesis inhibitor. Bevacizumab is also associated with a two-fold increase in the risk of arterial thromboembolic events, from 2.5% to 5%. These events consists primarily of acute coronary syndrome, transient ischemic attack, and stroke. Patients at risk for these events were those with a prior history of arterial thromboembolism and age older than 65 years.

VATALANIB

Vatalanib (PTK787/ZK222584) is a small molecule belonging to the phthalazine family of compounds. Initial testing determined that is a potent and selective tyrosine kinase inhibitor (21). Its predominant inhibitory effects are on VEGFR-1 and VEGFR-2, but it also has inhibitory effects on VEGFR-3, c-KIT and PDGFR-β. By inhibiting VEGF at the level of the receptor's tyrosine kinase, vatalanib disrupts downstream VEGF signal activation pathways. Single-agent studies suggested that PTK787 had modest single-agent activity in colorectal cancer; 8 of 18 subjects had stable disease by Southwest Oncology Group (SWOG) criteria. This has led to combination with chemotherapy in advanced colorectal cancer. However, in 2 phase III trials (CONFIRM-1 and CONFIRM-2), vatalanib, failed to improve response rates or progression-free survival when used in combination with FOLFOX4 (5-fluorouracil/leucovorin/oxaliplatin) compared with FOLFOX4 alone (22). These disappointing results have led to a closer examination of the pharmacokinetics of anti-VEGF small molecule inhibitors. Vatalanib, with a $t_{1/2}$ of 3-6 hours was dosed once every 24 hours in the CONFIRM-1 and CONFIRM-2 trials. Therefore, the ability of vatalanib to persistently inhibit the VEGFR-1 and VEGFR-2 TKs might have been depleted towards the end of the dosing period, and it is conceivable that intracellular VEGF signaling may have gone uninhibited for several hours.

CONCLUSION

This review showed the efficiency of new tagreted therapies in advanced colorectal cancer treatment. Application of certain agents has significantly prolonged time to progression as well as median survival of these patients. In the future, these agents used as monotherapy or incorporated in chemotherapeutical regimens, will certainly have a significant role in advanced colorectal cancer treatment, as well in other solid tumor therapy.

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