PhD THESIS
THE TARGETED THERAPY OF PROTEINKINASES IN CANCER

ABSTRACT

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Cancer is the world's second leading cause of death, surpassed only by cardiovascular disease. Cancer is characterized by uncontrolled cell proliferation and the absence of programmed cell death (apoptosis) which, with the exception of hematological cancers, generates an abnormal tumor mass. This primary tumor grows caused by the appearance of a new vascularization and, over time, acquires metastatic potential and spreads to other organs of the body, which causes metastases and eventually death. Cancer is caused by damage or mutations in cellular genetic material due to environmental or inherited factors. While surgery and radiation therapy are the primary treatment used for local and non-metastatic cancers, anti-neoplastic drugs (chemotherapy, hormone therapy, and molecular therapy) are the options currently used for metastatic cancer. Chemotherapy is based on inhibiting the division of fast-growing cells, which is a characteristic of cancer cells, but unfortunately also affects normal cells with rapid proliferation rates, such as hair follicles, bone marrow and gastrointestinal tract cells, generating the effects secondary characteristics of chemotherapy. The indiscriminate destruction of normal cells, the toxicity of conventional chemotherapeutic drugs, as well as the development of multidrug resistance, encouraged the need to find new targeted treatments against biological and simolecular changes specific to tumor cells. These new therapies have generated a great deal of interest, as evidenced by the large number of therapies approved by the FDA in recent years. Their mode of action is based on blocking the intracellular signal transduction pathways and / or cancer-specific proteins to induce cancer cell death by apoptosis and immune system stimulation, or by specifically delivering chemotherapeutic agents to cancer cells, minimizing unwanted side
In this paper, we selected for analysis three forms of cancer that are dependent on tyrosine kinase receptors, both in vitro and in vivo. Thus, both bronchopulmonary cancer and glioblastoma have subpopulations whose progression and aggressive phenotype are closely related to the epidermal growth factor receptor. Also, stromal gastrointestinal tumors are strongly influenced by the activity of the growth factor receptor derived from podocytes as well as by the c-KIT signaling pathway.

RESEARCH METHODOLOGY

APPROACH OF THE MAIN THEMES OF THE THESIS

OBJECTIVE NO.1. ASSESSMENT OF THE EFFECT OF EGFR INACTIVATION ON HGG TUMOR CELL VIABILITY

GBM are tumors with a wide range of genetic aberrations. The most commonly affected structure is EGFR, which has a mutant version and/or overexpression in over 50% of all tumors analyzed. Although the effect of EGFR inactivation has been studied in several clinical trials, no encouraging results have been obtained. In our study, we analyzed the effect of the EGFR inhibitor, AG556, on two cell lines immortalized by GBM (11 and 15).

OBJECTIVE NO. 2 EVALUATION IN A CASE STUDY OF IMATINIB TREATMENT (GLIVEC ®) FOR A PATIENT DIAGNOSED WITH A LOCALLY ADVANCED, INOPERABLE GASTROINTESTINAL STROMAL TUMOR.

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract, with an incidence of 3,000–5,000 cases per year in the United States. GIST arise from Cajal interstitial cells (ICCs), which play a particularly important role in modulating intestinal peristalsis. ICCs need the KIT receptor for normal development, but this activated receptor is the basis for tumor development in GIST.
In this study, our goal was to evaluate the response to treatment with Glivec® in a patient who has been on GIST for more than 10 years.

OBJECTIVE NO. 3 EVALUATION IN A CASE STUDY OF TREATMENT WITH ERLOTINIB FOR 10 YEARS, FOR A PATIENT DIAGNOSED WITH A STAGE IV B SQUAMOUS CELL BRONCHOPULMONARY CANCER

EGFR is a strong biomarker related to the appearance and development of NSCLC, having a strong influence on essential processes such as proliferation, inhibition of apoptosis, DNA synthesis, invasion and metastasis.

In this case study, we monitored the evolution of a 67-year-old man, recorded for 11 years (July 2009) with stage IVB bronchopulmonary neoplasm (brain metastases), former heavy smoker, with squamous histotype, treated for ten years with TKI Erlotinib in the second line, following progression to chemotherapy with Cisplatin and Gemcitabine.

OBJECTIVE NO. 4 EVALUATION OF THE EFFECT OF TEMOZOLOMIDE TREATMENT IN MONOTHERAPY ON A SHORT PASSAGE GBM CELL LINE

Current treatments for patients with GBM consist of surgery followed by radiation in combination with temozolomide. Despite therapeutic advances, the prognosis for patients with GBM remains bleak, with an overall survival of five years below 15%.

The aim of the current study was to analyze the effect of TMZ treatment on a GBM cell line in vitro.
RESULTS AND DISCUSSIONS

In our study, we found EGFR expression in two HGG cell lines. As in other studies published in this field, we observed that the amount of EGFR on the cell surface is correlated with the level of cytotoxicity induced by receptor inactivation (184). Thus, inactivation of EGFR by AG556 was more cytotoxic in cell line 15 which expressed higher levels of EGFR at the cell surface compared to cell line 11. Many other studies also found that targeted inhibition of a specific RTK is ineffective in the treatment of HGG, due to redundancy of the transduction signal and tumor heterogeneity. The ability to interrupt communication between common signaling pathways that are activated by multiple receptors is of paramount importance in the optimal development of TKI. In our previous studies, we found that simultaneous blockade of PI3K / Akt / mTOR pathways was more effective in destroying GBM cells than individual inactivation of PDGFR or VEGFR. Cell line 11 was previously studied in terms of intracellular signaling of tyrosine kinase receptors and was reported to have ligand-independent phosphorylation Akt, which was correlated with an increase in insulin-like growth factor-1 receptor inhibition resistance (117). Although the cytotoxic effect of AG556 in line 15 was greater than in line 11, it should be noted that the efficacy of this small molecule EGFR inhibitor as monotherapy in both cell lines was modest at best. Thus, the highest concentration of AG556 (30 μM) induced a cytotoxicity of approximately 17% at 3 days after treatment and prolonged exposure did not induce a greater cytotoxicity in cell line 11. In cell line 15, the cytotoxicity induced by 30 μM AG556 was 33% at 3 days of treatment and 44% at 7 days after treatment.

In our case study, we aimed to highlight how much Glivec® influenced the treatment response of patients with GIST. A 67-year-old patient diagnosed with unresectable GIST had a
bleak outlook at the time of diagnosis. Fortunately, due to the potent potential of Imatinib mesylate (Glivec®), MDT was able to control the disease for 10 years. Even when faced with an evolution of potential disease and the appearance of liver metastases, only by increasing the dose of TKI, MDT was able to stop the evolution of the tumor and maintain a satisfactory quality of life for patients who would previously receive a prognosis of survival. 6 months before the introduction of Glivec.

This 10-year survival is an exceptional achievement in terms of survival, disease control and quality of life for GIST patients. Glivec® has been shown to be extremely effective in the treatment of GISTs, showing strong disease-fighting potential while maintaining an acceptable level of toxicity and an overall good quality of life. This highlights the major impact this targeted treatment has had on cancer therapy, especially in the case of tumors that were previously considered untreatable.

In our case study, at the initiation of targeted molecular treatment, practice guidelines did not recommend testing EGFR mutational status and TKIs could be used in the treatment of any type of NSCLC, regardless of histotype or EGFR mutation status. The patient in our case study received treatment with Erlotinib in the second line of treatment following progression after chemotherapy-based treatment. It should be noted that prior to surgery, the number of invaded lymph nodes was not assessed by endoscopic ultrasound (EUS) or endobronchial ultrasound (EBUS). Also, no biopsy was obtained from the secondary brain determination, the MDT team considering that the risk to the patient was too high at that point. In this case study, the patient presented an interval without disease progression of 97 months, at the last CT investigation in May 2019, revealing the same residual lesions described above.

In this in vitro study, we chose to use a low-pass GB8B cell line. The results for TMZ treatment were similar to those obtained in the literature, with time and dose-dependent inhibition being observed.
CONCLUSIONS

Our study showed that cell lines responded differently to EGFR inhibition. EGFR inactivation by AG556 was more cytotoxic in cell line 15 which expressed higher levels of EGFR at the cell surface compared to cell line 11.

Customized analyzes are needed in patients with GBM to select the subpopulations that can benefit best from treatment with tyrosine kinase inhibitors.

Various factors, such as genomic instability, intrinsic radiological resistance, interactions between tumors and their microenvironment, mitogenic signaling pathways could be used in the development of targeted therapies and in the adaptation of current treatment approaches.

Glivec®-based treatment has been shown to be extremely effective in the treatment of GISTs, with strong disease-fighting potential, while maintaining an acceptable level of toxicity and an overall good quality of life.

The long-term response, low toxicity and acceptable quality of life underline the major impact that this targeted molecular treatment has had on cancer therapy, especially in the case of tumors previously considered incurable.

Our case study demonstrates that LC histotypes such as SCC, in which ESMO and NCCN do not recommend EGFR testing, may benefit from treatment with anti-EGFR TKI.
Due to individual variability and tumor heterogeneity, the status of the EGFR mutation should be analyzed in all patients with diagnosed NSCLC, not just in a few distinct subpopulations, where the benefit has been clearly demonstrated by clinical trials.

GB8B cells were sensitive to TMZ treatment, regardless of drug concentration and exposure time.

References

