UNIVERSITY OF MEDICINE AND PHARMACY
CRAIOVA
PhD SCHOOL

PhD THESIS
ABSTRACT

UPDATES IN MOLECULAR THERAPY OF SOLID TUMORS

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INTRODUCTION

1. STATE OF KNOWLEDGE

1.1. DIAGNOSIS AND TREATMENT OF SOLID TUMORS

Solid tumors have been slow in benefiting from new therapeutic options, and cancer treatment has been based for a long time on systemic cytotoxic agents. The last 20 years have marked an exponential development in the approach of cancerous disease so that new targeted therapies have begun to be used more and more.

Targeted therapies entered current medical practice about 15 years ago, with the findings of genetic mutations predicting the response to certain drugs such as tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC) or stromal gastrointestinal tumors (GIST) being of major importance in fueling further research. New therapies have dramatically altered survival data, one of the most representative being imatinib mesylate, which in 1998 marked a turning point in the therapeutic approach to malignant tumors, altering survival data in patients with GIST.

1.2 STROMAL GASTROINTESTINAL TUMORS

GIST tumor is the most common form of sarcoma, accounting for approximately 20% of all sarcomas and 1% of small bowel tumors. GIST tumors can occur anywhere in the digestive tract, most commonly in the stomach and small intestine, and much less often in the esophagus, rectal or abdominal cavity. In general, GIST tumors are aggressive tumors that have historically had a life expectancy of less than one year after diagnosis.
1.3 NON-MICROCELLULAR METASTATIC BRONCHOPUMONARY NEOPLASM

Also, the NSCLC treatment strategy has evolved from empirically administered chemotherapy to treatment personalization, based on histology and molecular markers. Therapeutic agents targeting driver mutations have revolutionized the therapeutic approach in the patient with metastatic NSCLC so that currently, a range of genetic tests outline the therapeutic profile of the metastatic NSCLC patient.

1.4 MULTIFORM GLIOBLASTOMA

Glioblastoma (also called glioblastoma multiforme - GBM) is the most common neoplasm of the central nervous system (CNS). It is also the most aggressive tumor form derived from glial cells, showing an impressive spectrum of genetic alterations, a major intratumoral heterogeneity and last but not least a high resistance to conventional therapies, with an unfavorable prognosis. The overall incidence is 2-3 new cases per 100,000 people / year in Europe and the United States. According to the United States Central Registry of Brain Tumors, GBM accounts for 14.9% of all brain tumors and 55.4% of all gliomas.

Currently, the therapeutic standard (SoC) for patients with GBM is surgical excision of the tumor with maximum safety margins, followed by concomitant chemoradiotherapy plus adjuvant chemotherapy with the alkylating agent Temozolomide (TMZ). Treatment in oncology has undergone drastic changes in the last decade, and these changes are unfortunately not reflected in therapeutic protocols targeting brain tumors. Despite multidisciplinary synergic strategies and individualized molecular therapies, the current treatment has a mediocre benefit, the prognosis of these patients being particularly poor, with a progression-free survival period (PFS) of 7-8 months,
an average survival (mOS) of 14-16 months and with a 5-year survival rate (OS) of 9.8%.

The complexity and genetic heterogeneity of solid tumors require further investigation of new methods that can predict the response to treatment, therefore, many studies have shown the importance of mathematical models that can help establish the prognosis and development of tumors.

We considered of great importance the understanding of the mechanisms that govern the development, the evolutionary process of the tumor, these being directly involved in the long-term control of the disease both in GIST as well as in glioblastomas and NSCLC.

PERSONAL CONTRIBUTION

2. RESEARCH METHODOLOGY

2.1 APPROACH OF THE MAIN THEMES OF THE THESIS

2.1.1 OBJECTIVE NO.1 TO COMPARE THE EFFECT OF IMMUNOTHERAPY BASED ON DENDRITIC CELLS AND VIRAL THERAPY WITH STANDARD TREATMENT. META-ANALYSIS STUDY

In recent years, immunotherapy has been the subject of many studies and offered different perspectives for the therapeutic management of high-grade glioma.

Our meta-analysis focused on the analysis of the effectiveness of dendritic cell therapy and viral therapy in clinical trials. 14 eligible studies were evaluated having as parameters both OS and PFS. The results from the experimental group were compared with those from the control groups.
2.1.2. OBJECTIVE NO.2 DEVELOPMENT OF A MATHEMATICAL MODEL TO PREDICT THE RESPONSE TO THE TREATMENT WITH THYROZINKINASE INHIBITORS OF A GLIOBLASTOM CELL LINE.

Researchers in several groups studied in vivo mathematical models to study the rate of growth and proliferation of gliomas. A mathematical model was developed for the growth of glioma and invasion of brain tissue, using the equation for the "rate of change in cell population density." In addition, mathematical models are important for understanding the biological mechanisms responsible for differences in the proliferative kinetics of patients with the same type of cancer.

In this study, we aim to build a mathematical model, focused on the interactions between the rate of proliferation, aggression and saturation of tumor cells. We used a cell line derived from a primary tumor (GBM). Based on the evolution of this cell line, we made a model analysis of the variables of the biological system.

2.1.3. OBJECTIVE NO. 3 EFFECT EVALUATION OF IMATINIB MESILATE THERAPY (GLIVEC ®) IN A PATIENT DIAGNOSED WITH AN UNRESECTABLE GASTROINTESTINAL STROMAL TUMOR.

GIST-type tumors are the most common neoplasms of stromal origin of the gastrointestinal system. About a decade ago, GISTs were considered virtually unaffordable from a therapeutic point of view, given the innate resistance to conventional treatments, such as chemotherapy or radiation therapy. The introduction of imatinib mesylate was the first and most important step in the treatment of GIST.
In this study, our goal was to quantify the impact that Glivec® can have on a patient suffering from an incurable oncological pathology.

**2.1.4. OBJECTIVE NO. 4  EVALUATION OF THE THERAPEUTIC EFFECT OF AN ANTI-EGFR THYROZINKINASE INHIBITOR IN A PATIENT DIAGNOSED WITH METASTASTIC BRONCHOPULMONARY CANCER, WITH SQUAMOUS HISTOLOGY**

EGFR is considered an important factor in the evolution of NSCLC, being incriminated in a multitude of cancer-related mechanisms, such as proliferation, cell cycle progression, DNA synthesis, invasion and metastasis.

In this study, we aimed to present the case of a 67-year-old man, diagnosed 11 years ago (July 2009) with stage IV bronchopulmonary cancer (brain metastases), a former heavy smoker with SCC tumor histology who was treated for ten years with erlotinib in the second line, at recurrence after first-line chemotherapy with a platinum doublet.

**2.1.5. OBJECTIVE NO.5  EVALUATION OF THE THERAPEUTIC EFFECT OF TEMOSOLOMIDE TREATMENT ON A GLIOBLASTOM CELL LINE**

TMZ is a second-generation oral alkylating agent widely used in the therapy of malignancies of the brain, including glioblastoma and astrocytoma in combination with radiotherapy or monotherapy. It penetrates the central nervous system, does not require liver activation to be effective and is generally well tolerated. As a chemical structure it resembles dacarbazine and is converted at physiological pH to the short-acting active compound,
monomethyl triazenoimidazole carboxamide (MTIC). In our experiment, we tested the ability of the alkylating agent TMZ to induce a cytotoxic response in the 18 HGG cell line.

3. RESULTS AND DISCUSSIONS

Cancer treatment is generally facing an upward trend in new therapies, which have produced significant improvements in patient survival. Unlike the steady pace observed in the development of new therapeutic agents for other forms of cancer, high-grade glioma (HGG) has remained particularly difficult to treat, with no notable improvements reported in recent years.

The current therapeutic standard, first established in 2005, has a low therapeutic index and has a large number of side effects. It consists of surgical resection, hyperfractionated radiotherapy with concomitant temozolomide (TMZ) followed by TMZ in adjuvant setting. Unfortunately, this combination showed only an incremental increase in survival and quality of life compared to previous therapeutic regimens (1).

The promising results of immunotherapy in other cancers and the high number of interactions between HGG and the immune system have highlighted the vast potential that this type of cancer has to offer.

Immunotherapy can be divided into passive and active.

Passive immunotherapy transcends the host immune system and involves the administration of antigen-specific monoclonal antibodies (Bevacizumab), cytotoxic immune cells such as cytotoxic T lymphocytes (CTL), or ex-activated lymphokine-activated killer cells (LAK), which are ex-vivo activated (2).

Active immunotherapy focuses on the interaction with the host's immune system to trigger an immune response. For example, peptide vaccination has a
mechanism similar to regular vaccination, based on the injection of specific tumor antigens coupled with highly immunogenic proteins in the hope of eliciting an immune response against the tumor (3).

There are also immuno therapeutic strategies that can be considered both passive and active. A good example would be viral therapy that uses viral agents to directly target tumor cells or as vectors for gene therapy, forcing the cell to express certain genes, making it susceptible to various treatment options (4, 5). The clinical impact of these new therapies was assessed by an inclusive meta-analysis of clinical trials compared to standard treatments.

### 3.1 STUDY NO. 1

Our study included a final number of 15 studies: 8 that used DC therapy, (6-13) 6 viral therapy (TV), (14, 19) only 1 adoptive therapy (20) and there were no studies for peptide vaccines and checkpoint inhibitors that meet the search criteria for inclusion. As there was a visible discrepancy in the number of studies for DC, viral therapy and adoptive therapy (21, 22 and 23 respectively), we opted to exclude the latter and discuss it separately. Finally, 14 studies addressed OS, while 7 addressed PFS.

Compared with standard therapy, our meta-analysis showed a 19% improvement in OS in patients receiving TV (HR, 0.81, 95% CI 0.71 0.91, p = 0.001), although there are a high heterogeneity (I² = 70%), confirmed by the x² test (Chi² = 16.71> 11.07). Regarding PFS, no improvement was determined (HR = 1.06, 95% CI 0.93-1.21), in addition to substantial heterogeneity (I² = 88%) and publication bias. Thus, the results were not statistically significant (p = 0.41).

Comparing DC and VT, we can conclude that DC vaccines are superior to VT in terms of both OS and PFS (35% improvement over OS and 41%
improvement in PFS for DC treatment), while TV did not show statistical significant results; \( p = 0.41 \).

In our experiment we established a mathematical model based on IC50 observed in an experimental framework for each therapeutic agent used on the GB10B GBM cell line. We compared the simulated results obtained using the mathematical model with the observed experimental value. A Pearson R value between 0.5 and 1 was considered to be a strong correlation between the experimental values and those obtained in the simulation.

### 3.2 STUDY NO. 2

In our experiment, Imatinib showed a cytotoxicity in the GB10B cell line of 5.5% for the minimum dose of 1 \( \mu \text{M} \), 16% for 2 \( \mu \text{M} \), 28% for 5 \( \mu \text{M} \), 40% for 10 \( \mu \text{M} \), 45% for and 20 \( \mu \text{M} \), and over 50% for 40 \( \mu \text{M} \) and 80 \( \mu \text{M} \). The mathematical model did not predict any cytotoxicity for doses of 1, 2 and 5 \( \mu \text{M} \), resulting in high residual values: -5.52 for 1 \( \mu \text{M} \), -16.64 for 2 \( \mu \text{M} \) and -28.34 for 5 \( \mu \text{M} \). The largest difference in cytotoxicity was observed for the 10 \( \mu \text{M} \) dose (60.2% observed compared to 98.36% anticipated), resulting in a residual value of -38.15. For the highest doses in our experiment, 40 \( \mu \text{M} \) and 80 \( \mu \text{M} \), the cytotoxicity induced by Imatinib was correlated with the values observed in the mathematical simulation: (46.2% vs 45.3%) and (42.2% vs 40, 2%) . Overall, the Pearson R value was 0.83, indicating a positive correlation between experimental and simulated values.

### 3.3 STUDY NO 3

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 proved efficacy of Imatinib mesylate (Glivec®), MDT was able to control the disease for 10 years. Even when faced with an evolution
of the 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 of 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.

3.4 STUDY NO. 4

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 metastasis, the MDT team considering that the risk for the patient was too high at that time. In this case study, the patient presented a progression-free interval of 97 months, at the last CT investigation in May 2019, revealing the same residual lesions described above.

3.5 STUDY NO. 5

In our experiment, we tested the ability of the alkylating agent TMZ to induce a cytotoxic response in the 18 HGG cell line. For the 200 μM TMZ
dose, the induced cell death rate compared to the control group was 23% after 24 hours (p≤0.05), 30% after 48h (p≤0.05) and 39% after 72h (p ≤ 0.05). For the 250 μM TMZ dose, the cytotoxic effect was 28% after 24h (p≤0.05), 35% after 48h (p≤0.05) and 43% after 72h (p≤0.05) in comparison with the control group. For the 300 μM TMZ dose, a more pronounced cytotoxic effect was observed, with proliferation decreasing by 24% after 24h (p≤0.05), 37% after 48h (p≤0.05) and 45% after 72h (p≤0.05) compared to the control group.

4. CONCLUSIONS

Patients who received dendritic cell-based treatment had significantly better median survival and a better disease-free period compared to patients treated with the current standard of care.

Patients who received treatment based on viral therapy had better median survival compared to patients treated with the current standard of care, the improvement of the disease-free period not being statistically significant.

Comparing dendritic cell therapy with viral therapy, we can conclude that dendritic cell vaccines are superior to viral therapy in both OS and PFS (35% improvement over OS and 41% improvement in PFS for cell treatment dendritic, while viral therapy did not show statistically significant results.

The mathematical model indicated a very strong correlation between the two sets of values, thus validating the value of the mathematical model in predicting the dose-dependent behavior of inhibitors used in preclinical experiments.

Given the accelerated evolution of translational medicine, it is necessary to develop as many mathematical models as possible to analyze the effect of compounds already validated for the treatment of some forms of cancer, in experiments on neoplasms with reduced treatment options.
Imatinib has been shown to be highly effective in our case study, leading to more than a decade of disease control without major toxicities and an increased standard of living for the patient.

Imatinib is an excellent example of how understanding intrinsic mechanisms that govern the evolution of a tumor can be the key to producing compounds that dramatically change the prognosis and quality of life of patients.

Erlotinib was shown to be effective in our case study, prolonging survival of a patient diagnosed with SCC for more than ten years, even if testing for EGFR mutation status is not recommended in this setting.

EGFR-TKI may play a role in the treatment of certain patients, and the status of the EGFR mutation should be part of the initial screening panel for each patient diagnosed with NSCLC.

In our study we observed that TMZ induced a decrease in the proliferation of glioma multiforme cell lines, this decrease being dependent on both the dose and the time of exposure to chemotherapy.
5. REFERENCES


16. Rainov NG. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical


