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ORIGINAL ARTICLE

A retrospective observational study on cases of osteosarcomas treated with a multitherapy: The rationale and effectiveness

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Abstract

BACKGROUNDS: The prognosis of patients with osteosarcoma in many cases remains poor, and life expectancy with lung metastases is around 12 months. Chemotherapy and radiotherapy can only temporarily control neoplastic progression, followed by developing chemo and radioresistant tumours.

METHODS: This is a retrospective observational study on 15 patients diagnosed with osteosarcoma and treated by a multitherapy approach. The multitherapy consisted of somatostatin and analogous (octreotide) all-trans-retinoic acid (ATRA), β-Carotene, axerophthol dissolved in vitamin E, vitamin D, vitamin C, melatonin (MLT), proteoglycans, glycosaminoglycans, hydroxyurea, and sodium butyrate.

RESULTS: This multitherapy increased the survival rate and life quality, without overt toxicity, compared to the standard treatment for osteosarcomas. The agents in this approach have several functions. They exert antiproliferative, antiangiogenic, cytostatic, antioxidant, antimetastatic, and immunomodulating features. Moreover, the inclusion of ATRA, MLT, and sodium butyrate has reinforced antitumor properties on cancer stem cells. Furthermore, the non-cytolytic and non-cytotoxic metronomic hydroxyurea dosage increased the biological therapy outcome by strengthening antitumor capability.

FINDINGS: This multitherapy approach is effective against osteosarcoma.

INTERPRETATION: The multistrategy of this multitherapy therapy are inhibiting the proliferative-invasiveness and neoplastic angiogenesis, silencing the survival system of cancer stem cells, enhancing the immunomodulatory and antioxidant activities, improving vitality and efficiency of normal cells, and depressing the efficiency and vitality of neoplastic ones.

Abbreviations:

ATRA	- All Trans Retinoic Acid	FGF	- Fibroblastic Growth Facto
CCK	- Cholecystokinin	GF	- Growth Factor
CSC	- Cancer Stem Cells	GH	- Growth Hormone
EGF	- Epidermal Growth Factor	GHR	- Growth Hormone Receptor
EGFR	- Epidermal Growth Factor Receptor	HGF	- Hepatocyte Growth Factor

IGF1-2 - Insulin-like Growth Factor 1-2
IGFR - Insulin-like Growth Factor Receptor
MLT - Melatonin
PDGF - Platelet-Derived Growth Factor
PRL - Prolactin
SST - Somatostatin
VEGF - Vascular Endothelial Growth Factor
VIP - Vasoactive Intestinal Peptide

INTRODUCTION

Osteosarcoma is an aggressive type of cancer with limited therapeutic responses to oncological protocols. Osteosarcoma is sporadic, idiopathic, and related to genetic defects and epigenetics. Genetic defects leading to osteosarcoma development are divided into simple or complex karyotypic defects. Simple karyotypic defects consist of disease-specific chromosomal translocations that lead to abnormal gene (and protein) functions, facilitating sarcoma development. Sarcomas associated with simple karyotypic defects include Ewing's sarcoma. Osteosarcomas with complex karyotypic defects may be secondary to radiotherapy (Arlen, 1971; Henderson *et al.* 2007; Hui, 2016). A study in patients diagnosed with radiotherapy-induced sarcoma showed that they are unique in their epidemiology and tumour characteristics.

Osteosarcomas are mainly treated with neoadjuvant chemotherapy, surgical resection and adjuvant chemotherapy. Radiotherapy is used less often and is generally applied when other treatments cannot achieve significant results (Mirabello *et al.* 2011; Rosenberg, 2017). The main site of metastasis in osteosarcomas is the lung. In metastatic disease, survival is generally poor. Thus, a new innovative strategy for treating osteosracoma is needed (Callesen *et al.* 2021).

Herein, we are presenting a retrospective observational study on osteosarcoma patients who received a multitherapy therapy (Di Bella method; DBM) protocol (Table 1). This multitherapy therapy consists of somatostatin and analogous (octreotide) all trans-retinoic acid (ATRA), β-carotene, axerophthol dissolved in vitamin E, vitamin D, vitamin C, melatonin (MLT), proteoglycans, glycosaminoglycans, hydroxyurea, and sodium butyrate. In this report, we are showing the effectiveness of our multitherapy compared to the standard therapy protocol. In addition, we are discussing the rational of each agent of our multi approach in fighting cancer cells and cancer stem cells (CSC) of osteosarcoma.

PATIENTS AND METHODS

Study design

This study is observational and retrospective study on fifteen patients with osteosarcoma. After giving each patient a summary about this treatment, each patient approved to enrol in the study and signed an informed consent.

Multitherapy Treatment: The Di Bella Method

The treatment applied is the Di Bella Method (DBM) on these patients include administrating several drugs, biologics, vitamins and supplements (Table 1). the Di Bella Method (DBM) was established (Di Bella *et al.* 1979a; Di Bella *et al.* 1979b). The DBM consists of administrating several specific molecules where each molecule is chosen based on its mechanism of action against tumor cells, CSC, proliferation and apoptosis, oncogenes, angiogenesis, molecular analysis, and genetic mutation. Besides, some molecules were chosen for their preservative mechanisms on healthy cells, including cell membrane integrity, DNA preservation, and mitochondrial function (Di Bella 2022).

Data collection

On the first visit, all medical history for each patient was collected, including diagnosis tests, images, and laboratory results. After the DBM was introduced, patients were monitored for several years, and data were documented.

RESULTS

Clinical Cases

We have treated 15 cases of different types of sarcomas: 6 patients with osteosarcoma, 4 with chondrosarcoma, 3 with Ewing's sarcoma, 4 with chondrosarcoma, and 2 with histiocytoma (Table 2). The stage and grade of each case at the time of diagnosis are presented in Table 2. Before starting the multitherapy, some patients had metastasis, relapses, or had recent surgery. Ten out of fifteen patients had stage IV disease, 2 had stage III, one patient had stage II, and two patients had stage I (Table 2).

DBM Therapy and Survival

After patients were treated with DBM, each patient was monitored for several years. Out of 15 patients, nine (60%) went into remission, one stayed in stable condition, and five had disease progression. Of the last five patients, three died (Table 2). Of the patients that died, one patient died within a year of starting DBM and had liver and lung metastasis. Another patient died after one year and already had a renal recurrence, and the third patient died after twelve years.

The survival data following DBM multitherapy showed that all patients with localized/regional disease survived more than five years compared to 77.5%, according to data from Surveillance, Epidemiology and End Results Program (SEER) (Table 3). Furthermore, the 5-year survival data for patients with metastatic disease and who had DBM multitherapy were 80% compared to 30.6%, according to data from SEER (www.seer.cancer.gov) (Fig. 1-2). Besides, patients treated with multitherapy, although experiencing rare and temporary modest toxicity, had a clear improvement in survival, objective response and quality of life.

Tab. 1. Multitherapy (DBM) protocol for patients with osteosarcoma

Medications	Chemical composition	Dosage	Method of administration	Frequency
Somatostatin	14-aa polypeptide	4 mg	Subcutaneous or preferably intravenous	Daily (12 night hours with infuser)
Octreotide LAR	Octreotide Acetate 8 aa	10 mg	Intramuscular	Weekly
Retinoid solution	All-Trans-Retinoic Acid Axerophthol Palmitate Beta-carotene Alpha Tocopheryl Acetate	0.5 g 0.5 g 2 g 1000 g	Oral	Daily (3 administrations)
Vitamin C	L-Ascorbic Acid	4 g	Oral	Daily (lunch and dinner)
Vitamin D3	1,25-diOH-Tachysterol	30 drops = 1 ml approximately = 1 mg	Oral	Daily (3 administrations)
Tetracosactide Acetate	Tetracosactide Acetate	0.25 mg	Subcutaneous	3 administrations per week, every other day, with infusion
Bromocriptine	Bromocriptine	2.5 mg	Oral	½ tablet twice a day
Cabergoline	Cabergoline	0.5 mg	Oral	½ tablet twice a week
Chondroitin Sulfate	D-glucuronic acid (GlcA) N-Acetyl-D-galactosamine (GalNAc)	500 mg	Oral	3 times a day
Glucosamine	D-Glucosamine	500 mg	Oral	3 times a day
Ursodeoxycholic Acid	Ursodeoxycholic acid	300-450 mg	Oral	Daily
Melatonin	Melatonin 12% Adenosine 51% Glycine 37%	100 mg	Oral	Daily
Sodium Butyrate	$C_4H_7NaO_2$	500 mg	Oral	2 times a day
Hydroxyurea	Hydroxyurea	500 mg	Oral	2 times a day
Calcium Carbonate	$CaCO_3$	500 mg	Oral	2 times a day
Calcium Levofolinate	Calcium Levofolinate Pentahydrate	22 mg	Oral	Once a day, every other day
Sucrosomial Iron	Sucrosomial Iron	14 mg	Oral	Once a day, every other day

DISCUSSION: THE RATIONAL

The present study demonstrates the efficacy of the multitherapy approach over the standard therapeutic protocols for osteosarcoma. This multitherapy uses agents to prevent cancer cell proliferation and components that induce CSC differentiation, immunomodulation, and have anti-oxidising functions. This therapy supports and enhances significant reactions and anticancer homeostasis, helping them counteract the onset of neoplasia and its progression (Di Bella, 2010). The DBM therapy pursues this objective through innovative formulations and criteria for the use of melatonin (complexed with adenosine and glycine), retinoids solubilised in vitamin E, and vitamins C, D3, and ECM components. Inserting polar components such as β-carotene and vitamin E between the phospholipids of a cell membrane stabilises it, preserving it from oxidative damage and free radicals (Shklar & Schwartz, 1996; Di Bella, 2005; Watters *et al.* 2009).

It isn't by chance that a significant percentage of aggressive bone sarcomas occurs in children and young people of prepubertal and pubertal age because the GH peak and negative melatonin peak coincide. Cells in the bone growth zones have the highest expression of the GH receptor. An increase in the incidence of osteosarcomas is also documented in taller-than-average subjects (Mirabello, 2011). There is clear and increasing confirmation of the primary role of GH in osteosarcomas (Mirabello *et al.* 2011). The latter study documented that subjects with high birth weight (≥4,046 kg) had an increased risk of osteosarcoma compared to average birth weight (2,665-4,045 kg) (OR 1·35, 95% CI 1·01-1·79). Taller-than-average individuals (51-89th percentile) and very tall individuals (≥90th percentile) had an increased risk of osteosarcoma (OR

Tab. 2. Osteosarcoma patients demography, diagnosis time, and clinical conditions with standard treatments and the multitherapy approach

Anagraphic		Anamnesis								Multitherapy - Treatement period and result				
File no.	Date of birth	Diagnosis	Diagnosis date	Stage	Grade	Interventi Chirurgici	CT	Group	DMB	Conditions upon arrival	Stage	Result	Efficacy	Current Conditions
N 1	28/06/1990	Osteosarcoma	25/09/1995	II B	G 4	1996 - bone resection	yes	E	1998	pulm. nodules - cardiopathy caused by ct	IV B	Remission	R	in 2007 was in remission
N 2	04/04/1966	Osteosarcoma	09/09/1997	II B	n.d.	01/01/1998	yes	E	1998	After surgery	II B	Complete remission	R	Absence of disease
480	19/03/1999	Osteosarcoma	01/09/2003	II B	G 3	thigh (2003) lung (2005)	yes	E	2006	Relapse with lung metastases	IV B	Complete remission	R	Absence of disease
33	05/02/1940	Osteosarcoma	05/09/1997	III	G 4	thigh 1997	yes	E	2004	Lung metastases	IV A	Complete remission	R	Absence of disease
42	06/07/1967	Mesenchymal chondrosarcoma	08/05/1997	III B	G 3	surgery in 1997/99 - relapsed in 2007 - relapsed in 2009	yes	E	1998	After the first surgery	III B	Remission/ Progression	RP	Deceased 10/2010
1716	24/02/1952	Histiocytoma	1996	II A	G 3	1996 - 2001 (replase)	no	D	2001	Relapse	III	Complete remission	R	Absence of disease
1718	16/08/1930	Malignant fibrous histiocytoma	ott-2000	I	G 2	Radical resection	no	C	2001	After surgery	I	Complete remission	R	Absence of disease
368	03/09/1988	Bilateral chondrosarcoma	mar-2002	III	G 3	2000-2001-2002-2005	no	C	2005	Cranial recurrence - suspension of treatment	IV	Stability	S	Stability
6640	23/11/2003	Ewing's sarcoma	25/01/2012	II	n.d.	Surgery+RT+CT	yes	E	2015	Lung metastases	IV	Absence of disease	R	Absence of disease
10457	30/10/1985	Ewing's sarcoma (PNET)	10/04/2019	IV	n.d.	Surgery + CT	yes	E	2019	6 months after surgery	IV	Partial remission	R	Reduction of nodules
11115	29/07/1977	Chondrosarcoma	13/09/2016	I	G 2	Surgery + RT	no	D	2020	Absence of disease	I	Absence of disease	R	Absence of disease
4345	16/06/1954	Chondrosarcoma	01/10/2001	I	n.d.	multiple	no	C	2012	After several relapses	IV	Progression	P	Progression
4440	06/06/1994	Osteosarcoma	01/01/2002	I	manca	2002	yes	E	2012	Renal recurrence	IV	Progression	P	Deceased 2013
7603	30/05/1967	Sternal osteosarcoma	01/01/2013	IV	manca	Surgery	yes	E	2016	Lung and liver metastases	IV	Progression	P	Deceased 2016
9093	22/09/1975	Ewing's Sarcoma	11/04/2008	I	manca	Surgery + CT in 2008	yes	E	2017	Relapse of cranial Ewing's sarcoma	IV	Progression	P	

Legend of groups and efficacy "Group A"= no previous treatment; "Group B"= CT/RT; "Group C"= surgery; "Group D"= CT + RT; "Group E"= surgery + CT/RT. Efficacy: R= remission; S= stability; P= progression.

Tab. 3. Survival patients with osteosarcoma treated with multitherapy

GENERAL SURVIVAL	1 year	5 years
(obs - rel)	100%- 100%	93% - 95%
SURVIVAL (OBS) COMPARED TO ARRIVAL STAGE	**1 year**	**5 years**
Localized / Regional (5)	100%	100%
Metastatic (10)	90%	80%

1·35, 95% CI 1·18-1·54 and OR 2·60, 95% CI 2·19-3·07, respectively; $P< 0·.0001$) (Mirabello *et al.* 2011; Di Bella *et al.* 2018). Besides, the significant decrease in melatonin after 3 to 5 years of age coinciding with a GH peak increase is wildly documented in children with osteosarcoma (Cavallo, 1993; Lu *et al.* 2019).

Protein synthesis and cellular proliferation are closely dependent on the interaction of prolactin with GH (Lincoln *et al.* 1998; Friend, 2000; Barnett, 2003), and on mitogenic molecules, GH-dependent growth factors that are positively regulated by it, such as EGF, FGF, HGF, IGF1, VEGF, PDGF as well as gastrointestinal growth factors such as VIP and CCK (Murray *et al.* 2004; Hagemeister & Sheridan, 2008). The GH and PRL receptors are co-expressed on cell membranes and dimerise, amplifying the transduction of proliferative signalling pathways (Kelly *et al.* 1993). Numerous studies indicate how these pituitary hormones play a crucial role in the development and progression of human tumours. Their receptor expression is ubiquitous (Batra *et al.* 1997; Cameron *et al.* 1979) and particularly high in cancerous tissue. A dose-dependent relationship between GH-PRL receptor expression and tumour induction and progression processes is detected histochemically and through immunohistochemistry techniques, Western Blot, in situ hybridisation, and qPCR techniques. The documentation of much higher GHR concentrations in tumour tissues compared tonormal and peritumoural tissues confirms its powerful mitogenic role (Lincoln *et al.* 1998; Friend, 2000).

In various sarcomas, the increasing percentage of tumour stem cells compared with these tumours' different neoplastic phenotypes is most likely the main reason these tumours rapidly acquire resistance to chemo and radiotherapy, become very aggressive, and progress rapidly. Furthermore, understanding the biology of sarcoma stem cells improved DBM multitherapy (Hatina *et al.* 2019). For these reasons, we have gradually increased the doses of molecules documented

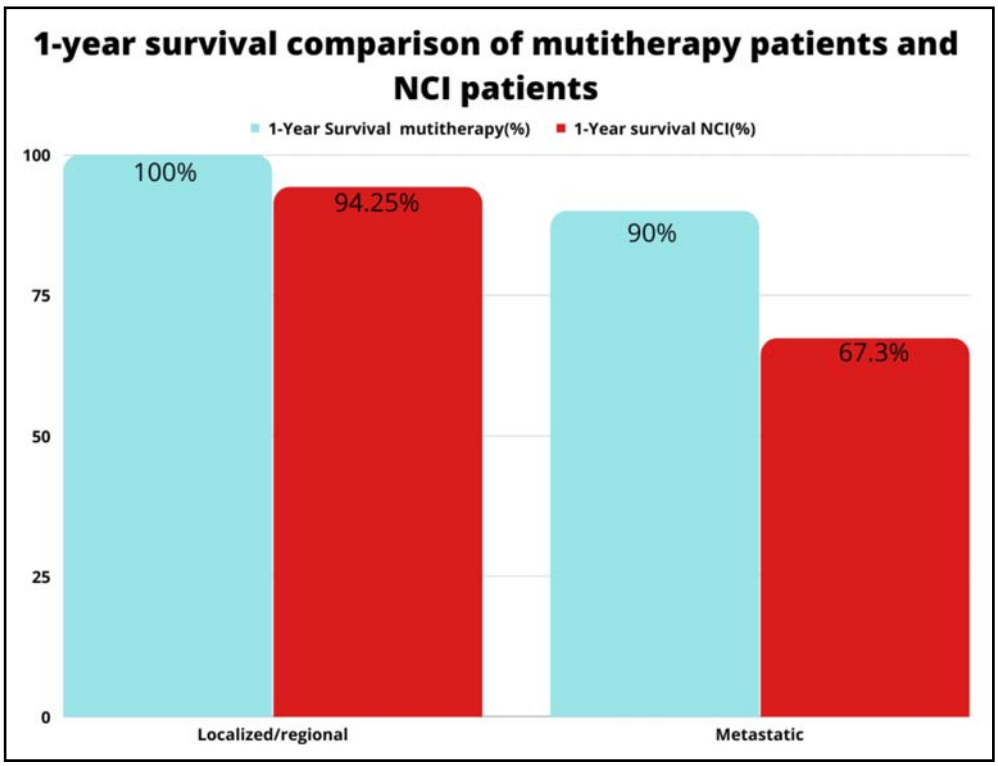

Fig. 1. Comparison of 1-year survival of osteosarcoma patients treated with multitherapy and other cancer treatments (data collected by the National Institute of Cancer). This graph shows that the survival of multitherapy patients is considerably greater than the data reported by NCI.

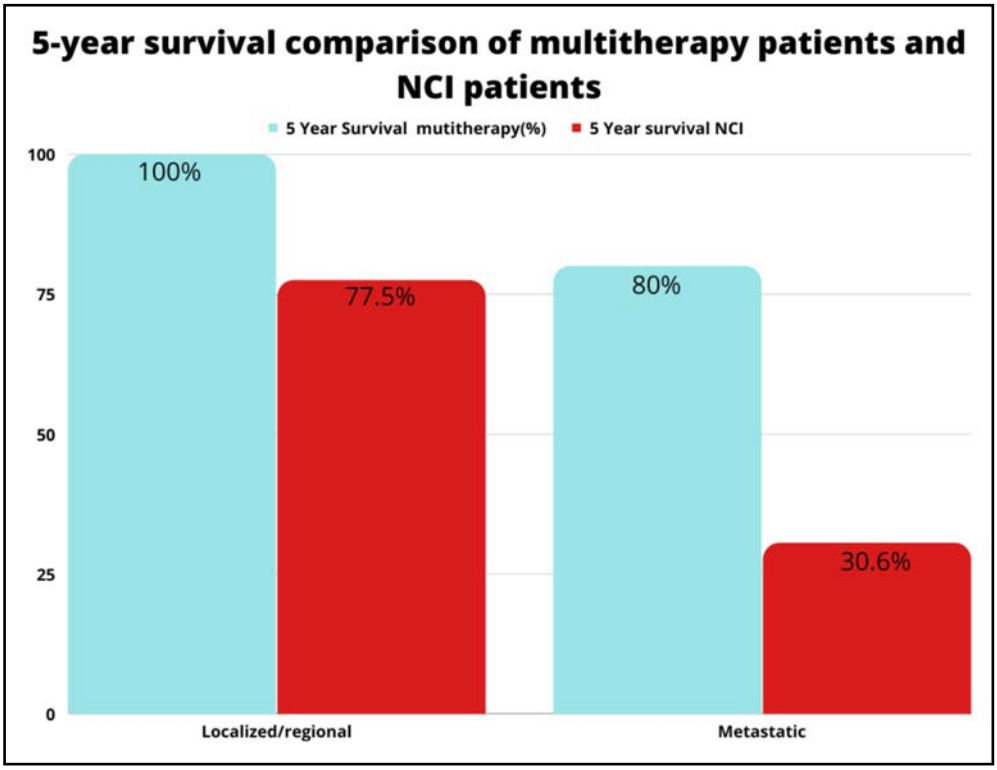

Fig. 2. Comparison of 5-year survival of osteosarcoma patients treated with multitherapy and other cancer treatments (data collected by the National Institute of Cancer). This graph shows that the survival of multitherapy patients is considerably greater than the data reported by NCI.

in the scientific literature to negatively regulate CSC, such as MLT, ATRA, and glucoasmine which improve the differentiation and reprogramming of tumour stem cells by negatively regulating proliferation, invasiveness, and resistance. Furthermore, modifying the criteria and methods of administration of alkylating drugs, such as hydroxyurea, allowed better control of the proliferation and invasiveness of tumour cells (Di Bella & Gualano 2006; Di Bella *et al.* 2013; Di Bella L & Di Bella G, 2015).

The concomitant administration of sodium butyrate creates an epigenetic context of chromatin relaxation, essential for the interaction with transcription factors of the zinc finger and homeodomain family, the RXR, VDR, RZR, ROR receptors, co-expressed on nuclear membranes and involved in differentiation processes. The differentiating components of multitherapy, such as the solution of retinoids in vitamin E, vitamins C, D and MLT counteract the mutagenic capacity of cancer cells based on cancer cells defence system and cancer cells survival programme. The first forms of life, prokaryotes, survived to the present day because, as they evolved, they became equipped with a defence system based on a programme of mutations, which allowed them to repair DNA damage caused by various adverse events. The prokaryotes transmitted the survival programme to bacteria, which in turn transferred it to somatic cells. Radman has identified and studied this programme of survival and defence transferred from prokaryotes to eukaryotes, and from the latter to somatic cells. Because of its functions and survival purpose in emergency conditions, he called it the "SOS programme", which somatic cells access to overcome critical situations (Radman, 1975). Tumour cells in an acute stress situation implement DNA repair systems and express or silence genes according to their needs, selecting and retaining for each mutation a series of advantages much more quickly and efficiently than bacterial cells. Israel (1996) studied the SOS system, identifying numerous gene homologies between neoplastic and bacterial cells. The SOS system allows neoplastic populations to become progressively refractory to various oncotherapeutic treatments through DNA repairs and genetic recombinations.

In the human body, under stable conditions and biologically balanced, the SOS system is silenced and inactive, blocked by a transcriptional repressor, the LEX-A protein. When the DNA of a somatic cell is severely damaged, to access the SOS survival path and repair the DNA, the cell deactivates the LEX-A transcriptional repressor using the REC-A positive regulator. The expression of SOS thus initiates a series of mutations that repair but at the same time modify the DNA, initiating the carcinogenesis process. The mutated cell begins a tumoural involution, continuously selecting and retaining, with a progression predefined by the SOS programme as confirmed by Lambert *et al.* (2011). More recently, other including Russo *et al.* (2019) showed that various mechanisms against tumour

cells, even beyond chemo- and radiotherapy, such as monoclonal antibodies and inhibitors of ligand mitogenic signalling pathways such as EGFR, VEGF, IGF1, FGF, etc., can rapidly activate the SOS system and large number of multiple survival mechanisms.

CONCLUSIONS

This study analyzed 15 cases of osteosarcoma treated with multitherapy. The treatment significantly improved the survival and quality of life in sarcoma patients. The multistrategy of this multitherapy therapy are a) inhibiting the proliferative-invasiveness and neoplastic angiogenesis, b) silencing the survival system of CSC, c) enhancing the immunomodulatory and antioxidant activities, d) improving vitality and efficiency of normal cells, and e) depressing the efficiency and vitality of neoplastic ones. Thus, the multitherapy extends the activity to fight multiple vital reactions of the neoplastic biology and shifts the therapeutic axis from a pure cytotoxic-cytolytic concept and the illusory and utopistic eradication of all cancer cells, to the gradual physiological reconversion of the vital functions deviated by cancer, balancing immuno-neuro endocrine homeostasis, and reprogramming cancer stem cells tofull differentiation.

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