NEW PROSPECTS FOR THE THERAPY OF THE PATIENTS WITH DISSEMINATED MAMMARY CARCINOMA

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We proved 50% antitumorigenic activity of RL-175 preparation in 40 patients with disseminated breast cancer, when modern physical-chemical therapy had failed or when the patients had been rejected treatment in view of their high resistance. In monotherapy RL-175 surpasses cytostatic agents known in literature both itself and in combination with the other agents. The RL-175 effect appears to be mediated by the increase in DNA reparation mechanisms and the activation of genetic mechanisms of apoptosis program, as well as the enhancement of cell genes transcription control.

In oncology the prospects of RL-175 as the means of last hope is accounted for not only dramatic tumor regression, when taken by the patients with advanced breast cancer (50% and more), but also concomitant 90% relief of pain syndrome, extremely low toxicity, and high efficacy in the prophylaxis of this severe disease.

Introduction

It seems that cell malignancy, in human breast, in particular, is caused by multiple factors such as hereditary mutations, damage of different genes BRCA1, BRCA2, mts1, CYP19, polymorphism of steroidogenesis genes and the other damage of genome still unknown [1-4]. For example, the experiment proved an important role of an mts1 truncated gene deprived of a segment of 450 nucleotides in the progress of breast cancer. CYP19 aromataze gene, which is localized on the long arm of the 15th chromosome, involves several non-complied variants of exon I into expression. Due to the existence of multiple promitors of CYP19 gene transcription and depending on their disposition across each variant of exon I and II, the structure of the transcript is characterized by the tissue specificity.

However, in spite of considerable progress in molecular-genetic methods of the analysis of nucleotide sequences of DNA and RNA, the exponential growth of data on mutations at the stage of replacement or deletion of nucleotides, the structure of chromosomes and other biological systems, morbidity and mortality rate after breast cancer in the world tends to grow [5,6]. Such situation in oncology has developed due to the absence of a reliable strategy of search of effective drugs for the treatment of patients with disseminated mammary carcinoma.

By now, capecitabin, docetaxcel, doxorubicin, paclitaxcel, vinblastin, and other agents have demonstrated the most significant effect (> or = 50%) on the regression of disseminated mammary carcinoma in monotherapy. A large number of cytostatic agents make an imperial effect of less 50% (5-phtoruracilum, cyclophosphamide, methotrexatum, thiophosphamidum, vinblastin, vincristine, chlorbutinum) [7]. The basis of any chemotherapy program for disseminated mammary carcinoma is the combination of cytostatic agents. One of the most effective combinations is docetaxcel, 5-phtoruracilum, cyclophosphamide, methotrexatum, doxorubicin, and paclitaxcel.

The effect of the above combination of cytostatic agents during the first stage of chemotherapy is 50% and more in patients with disseminated mammary carcinoma. However, only 5 to 10% patients demonstrate total tumor involution. During the third and the later stages of the process the total therapeutic effect (system response) doesn’t exceed 10 to 15%. [7]

During the later stages the effect of the mentioned cytostatic agents falls to zero level of
the objective response. Patients with remote metastases of $T_{3-4} N_1 M_0$ and $T_{3-4} N_1 M_{1-3}$ stages appear to have irreversible morphologic and metabolic changes. This patient population with an early fatal prognosis lacks reliable strategy of both improving survival in general and restoration of acceptable quality of life. For the majority of these patients recurrence therapy is an extremely complicated problem, since their reserves have already been exhausted at the stage of primary treatment.

In this article we submit the results of clinical tests of a new generation anticancer agent RL-175 in 32 volunteers with fatal disseminated breast cancer of $T_{3-4} N_1 M_0$ and $T_{3-4} N_1 M_{1-3}$ stages. By the authority of the Ministry of Public Health of Russia № 10-13, October 27, 1991, patients who had previously undergone a complete course of conventional physicochemical therapy, were included in the study. In these patients standard treatment was not only inefficient, but provoked metastasis processes. Eight other patients of different age took part in the drug test. They had breast cancer of stages III and IV detected for the first time and showing quick progress.

Patients and Methods

40 hopeless patients of aged 30 to 61 years old (average 38) with disseminated breast cancer underwent treatment with RL-175 within the period of 1988 to 2000. 32 of them had undergone complete courses of up-to-date therapy (surgery, radiation therapy, hormonal therapy and chemotherapy) in different specialized clinics and centers in the USSR and Russia.

The patients were divided into 4 groups depending on the peculiarities of the clinical pattern. Group I included 4 patients aged 30 to 45 years in pre-menopausal period and 4 elderly patients (45 to 61 years) in a deep menopause and breast cancer of stages III and IV detected for the first time and growing progressively worse very quickly. These patients refused to undergo the conventional chemotherapy or gamma-ray teletherapy, neither mastectomy because of high resistance to the previous treatment. 32 patients with disseminated breast cancer of $T_{3-4} N_1 M_0$ and $T_{3-4} N_1 M_{1-3}$ stages took part in the study of groups II-IV. In these patients the conventional radiation, hormonal and chemotherapy turned to be unsuccessful. These groups of patients had metastases found in soft tissues, lymph nodes, and bones, especially thoracic and lumbar vertebrae.

The following factors served as the inclusion criteria: voluntary consent of a patient to sustain treatment, strict verification of the diagnosis, objective assessment of the patient’s status, extremely low effectiveness of the conventional cytostatic agents. Also, the ethic norms and claims made to the adaptive immunotherapy of cancer (Rosenberg [8]), as well as the standards worked out by the Helsinki Declaration of 1975 (revised in 1983 and 2000) were taken into account. Patients who died before the end of the complete course of treatment and patients with irreversible associated pathology were excluded from the study.

The objective factors testifying to the beneficial antitumor effect of RL-175 as of the only treatment of the last line of chemotherapy were: a 50% regression of tumor and metastases; an average three-year recurrence-free life span; almost complete relief of pain syndrome; and rehabilitation of subjective and objective parameters and life quality. Before treatment almost all patients had suffered severe or very severe pain (the third step of the analgesic ladder) and for this reason they were given intramuscular injections of opiates (omnoponim, morphini hydrochloridum) 2 to 4 times a day. The life quality was assessed by the relief of pain syndrome, day activity, duration of night sleep and so on.

RL-175 was synthesized by the author of this paper. We conducted the pre-clinical trial of RL-175 at the Oncologic Scientific Center of the Academy of Medical Sciences of Russia (Moscow) and other academic
centers in Russia. Physicochemical and biological properties of the preparation are described in the works by Lokhov [9-13]. The drug showed low carcinogenic and mutagenic activity in experimental models (rats and mice). LD\textsubscript{16} for RL-175 constituted 1200 mg per 100 g of the body weight when introduced per orally.

All the patients were given RL-175 daily per orally in the dose of 20 mg per 2 ml of 30% alcohol for 30 days (the first course). The second course was performed after a five-day interval. The duration of the third course depended on the objective and subjective status of the patient. Anti-tumor effect of RL-175 in groups 1-4 is shown in tables I-IV.

**Results**

40 patients with disseminated breast cancer were divided into 4 groups depending on the peculiarities of the clinical course. The protocols of treatment with only RL-175 are shown for each group. The efficacy of the drug in patients with disseminated breast cancer was \(\geq 50\%\).

**Group I.** Patient B (b. 1959) fell ill at the age of 32. Histological and mammographic diagnosis was breast cancer, nodose form of T\textsubscript{3}N\textsubscript{1}M\textsubscript{0} stage. The tumorous focus with radial outline on the mammogram of 20 May 1992 (Figure 1) was a centrally located node of homogeneous density of 4.0 x 7.0 cm and of irregular round shape. The metastases were discovered in only one axillary lymph node.

![Figure 1](image.png)

**Figure 1.** Patient B. Mammogram: a centrally located node of homogeneous density of 4.0 x 7.0 cm, irregular round shape, and indistinct outline. In three months tumor regression to 3.0 x 2.0 cm (78.6\%) was registered.

This patient refused from chemotherapy, gamma-ray teletherapy, or mastectomy. She only underwent a thirty-day course of monotherapy with a total dose 0.4g of RL-175. The comparison of the mammograms made before treatment on 20 May 1992 and after treatment on 20 Aug 1992 showed that the size of the intensive shadow in the central parts of the tumorous focus with radial outline (due to the skin thickening) decreased to 3.0 x 2.0 cm (\(\geq 70\%\)). The individual status of the patient improved considerably. The anesthetic effect predominated in all 8 patients of this group on day 10. As a result, these patients refused from taking non-steroid analgesics. The duration of the night sleep and the day activity increased evidently. The second biopsy №027927 was taken on 18 Sept 1992. Two clusters of epithelial cells with a dramatic fat degeneration proved to reveal...
the presence of the complexes of medullar carcinoma.

According to the data of Bernstein et al [4], tumor regression of similar localization has been controlled by glucocorticoids. Hence, the mono-regime effect of RL-175 seems to be affected by age-related changes of sensitivity of adenohypophysis. In fact, 4 elderly patients of this group (45 to 61 years) with a deep menopause did not appear to show any clinical effect even after 3 courses of treatment.

**Group II.** Patient P (b. 1951) was under medical observation at the regional narcological dispensary in Vladivostok from 20 Aug 1993 for disseminated breast cancer of T3N2Mx stage. Histological diagnosis was high-grade differentiated adenocarcinoma with metastases to the thoracic part of spine Thx-Thx1 and a severe pain syndrome. The patient underwent the courses of gamma-ray teletherapy, combined chemotherapy and hormonal therapy. Radical bilateral ovariectomy was performed on 10 Dec 1993. However, shortly after surgery the patient suddenly worsened and was discharged from the hospital. Supportive therapy was ineffective.

After thorough re-examination, the patient underwent 3 courses of monotherapy with RL-175 (18 Feb to 20 Mar 1994; 12 Jul to 14 Aug 1994; 10 Nov to 10 Dec 1994). In 1995 the patient underwent control check up. Mammography showed that from 8 June 1994 breast tumor decreased by more than 50% (**Figure-2**), infiltration also decreased, whereas fibro-regenerative manifestations increased. Total relief of the pain syndrome was achieved. The patient started leading active life without any limitations. Judging by the subjective and objective parameters, the recurrence-free interval in this patient lasted 4.5 years.

Out of 16 patients of this group, 10 had their recurrence-free interval from 3 to 4.5 years.

**Group III.** Patient S. (b. 1944) was registered at Uman oncological dispensary (Ukraine) on 20 Jan 1989 with the diagnosis of disseminated breast cancer of T2N2M1 stage with the metastases to the thoracic spine Thx. Radiography showed that thoracic spine Thx was almost completely destroyed. The patient underwent Halsted radical mastectomy on 18 Jul 1989. Then a complete course of combined chemotherapy was carried out. However, after the mastectomy metastatic progress was detected. Severe pains appeared. The patient was bedridden. She had to wear a corset. Severe intoxication developed, and the patient took steroid analgesics. She worsened dramatically and poor outcome was predicted.

The patient underwent only 2 courses of RL-175 (17 Feb to 14 March 1991; 17 March to 12 Apr 1991). During the first course reliable pain relief was marked. After treatment stable radiological remission of more than 60% and partial restoration of bone structure Thx and Thx1 were achieved. Active unrestricted life style was also restored. She put off her corset and took up her previous job. The follow-up period was 42 weeks. Insignificant progress of metastatic damage of Thx and Thx1 was detected in 12 months (**Figure 3**). Another patient with similar history had a fracture of thoracic ThX-XI and lumbar L1-II vertebrae and total loss of mobility.

Patient G-H underwent 2 courses of RL-175. The anesthetic effect became clearly evident on days 10 to 13. She could do some sedentary work and in 1 to 1.5 months walk and do some work about the house. She took off her corset. A recurrence-free period of stable roentgenologic regression of tumor focuses was 42 months. 7 of 12 patients with disseminated breast cancer demonstrated the same interval with 50 to 70% positive results.
Figure 2. Patient P. Mammogram: high-grade differentiated adenocarcinoma with metastases to the thoracic part of spine ThX, ThXI of irregular radiant form and unclear contour. Tumor regression is more than 50%. Tumor size progress has not been identified within 44 months.

Figure 3. Patient S. Roentgenogram of the thoracic spine of 13 Dec 1990 (a). Pathologically almost a compression fracture of vertebrae ThX and ThXI. Roentgenogram of the same segment of spine in four months (b). Almost total tumor regression is observed. Metastatic lesion of ThX and ThXI is insignificant 15 months after the first course of treatment, 24 Mar 1992 (c).
**Group IV.** Patient E (47 years) first turned for consultation to Vladikavkaz oncological dispensary in January, 1987. In May, 1987 she underwent Halsted radical mastectomy. In March, 1989 histological and roentgenological evidence of a tumor focus in the right mammary gland was discovered with metastases into lymph nodes and metastatic damage of the 8th left rib with its total destruction. Patient E. underwent three RL-175 courses in mono-regime. After the first course, metastasis stabilization, partial restoration of the affected rib structure (Figure 4) and regression of the process in lymph nodes were identified. The patient was under medical supervision at home in the regime of supporting therapy for 38 months. All 4 patients of the group showed reliable pain relief and increase of sleep duration.

Of 4 patients of this group only 2 demonstrated about 50% general improvement after treatment.

![Figure 4](image.png)

**Figure 4.** Patient K. Metastatic lesion of the 8th rib on the left with a complete destruction of the back segment is found on the diagnostic chest roentgenogram of 29 Mar 1989. Eight months later bone structure of the affected rib was partly restored along the back axillary line.

**Discussion**

We have shown the clinical outcomes in groups I to IV of patients of pre-menopause age suffering from disseminated breast cancer, in whom the standard treatment had been completely exhausted. Basing on the results given above, we can conclude that RL-175 antitumorigenic activity considerably higher, than that of all the other cytostatic agents and drugs described in literature, both in mono-regime and combined with the other known drugs on all the lines of chemotherapy.

Nowadays, docetaxcel of taxan row is considered the most effective preparation for disseminated breast cancer. Numerous randomized investigations in patients with disseminated breast cancer showed that after docetaxcel therapy the frequency of objective response (complete and incomplete regression) as the first line of therapy was more than 60% [7]. Multiple studies performed in 30 oncological centers of the world showed that an objective effect of docetaxcel as the chemotherapy of the second line was 41% with median duration of its effect amounting to 6 months [14]. The other taxan, paclitaxcel used for chemotherapy of the first line in patients with disseminated breast cancer showed 29% to 62% improvement is from with median duration of positive response of 6 to 8 months [15].

Of the new generation of cytostatic agents vinorelbin (navelbin) of vinkaalkaloids has to be mentioned. Its effect in the first line therapy of disseminated breast cancer reaches 35 to 65% with median duration of 5 to 9 months [16], and in the
second line therapy 20 to 31% with median duration of 4 to 8 months.

New drugs (such as taxans, vinorelbine, kapecitabin) enabled the development of highly efficient combinations. Thus, the combination of docetaxel and doxorubicin in the first line therapy of disseminated breast cancer resulted in about 82% objective response. In the later randomized investigations of the same drug combination the objective response in patients with disseminated breast cancer was higher than 60% and 47% [7]. The combination of doxorubicin and paclitaxel allowed for 94% increase in objective response rate [17].

However, in the third and the later lines of therapy, the percent of clinical effect of the known drug combinations dramatically decreases to 10 to 15%. In comparison, we are demonstrating here that the objective positive effect of RL-175 in mono-regime for disseminated breast cancer increases from zero to 50% and more.

It should be stressed that the known cytostatic agents display considerable hematological toxicity both in mono-regime and in different drug combinations. Moreover, treatment with the above combinations is accompanied by the development of chronic cardiac toxicity in 18% cases. RL-175 is among the powerful anti-anemic factors of blood haemopoiesis regulation [13]. It also significantly influences the regulation of energy homeostasis [9–13].

At the same time, median duration of the objective response for the known cytostatics both in mono-regime and combination as chemotherapy of the first line constitutes 6 to 8 months and on the last line decreases to zero level, whereas our clinical data in groups I to IV of patients with disseminated breast cancer demonstrate this median increase up to 3 years and more. The given comparison by the duration median of the recurrence-free interval of therapy is extremely important for understanding the fact that the known strategy of search for the drugs with selective antitumor action on specific targets of malignant cells is of low efficacy. We believe that this is due to multivariant transmission of signals for tumorigenic growth into the cell. The new conception of search for highly efficient antitumor agents and methods for treatment of patients with disseminated breast cancer are described below.

ATP concentration in tumorigenic cells near plasma membranes falls to zero, and this is the reason for the damage of the control of complex interaction of regulator proteins (c-myc and p53) which are the factors of transcription. At the same time, a tendency to local synthesis and heterogeneous ATP accumulation in plasma membranes of tumorous cells has been revealed [18]. These locally formed and short-living ATP molecules can activate tyrosine kinases and then through phosphorylation by tyrosine residua, launch the cascade multivariant mechanism of tumor growth by tumorigenic peptides [19].

Our clinical results on tumor regression in patients with disseminated breast cancer can be interpreted from the point of view of inhibition by RL-175 preparation of the mechanisms of perception and transmission of signals of tumorigenic factors of growth into the cell.

On the other hand, the analysis of literature on the influence of different inhibitors of tyrosine kinase receptors on ATP, insulin, and other activation factors reveals a low value of the above mentioned anticarcinogenic conception. Chiefly, it is because the processes of perception and transmission of signals for tumorigenic factors of growth into the cell are miscellaneous, multivariant, and very often depend on a variety of internal and external factors [14].

P53 gene is the sensor of DNA damage and cell cycle disorder. P53 mutation is found in more than a half of the cancers, growing during prolonged chemotherapy [20]. It was shown that in case of P53
activation, the gene protein can initiate the two following independent programs [20]:

- Temporary stoppage of the cell cycle in G$_1$S-phase of kariomitotic cycle by means of P21$^{\text{WAF1}}$ protein inhibiting cyclin-dependent kinases;
- Inhibition of the launch of apoptosis program with the aid of Bax or Bid genes inactivation, proapoptosis genes of Bcl-2 family.

In literature [2, 20] there are also the data on participation of P53 in the processes of DNA reparation through activation of gene P53R2 encoding ribonucleotide reductase. The decrease of gene P53 activity or mutation resulting in the loss of the ability to initiate apoptosis is a serious factor predisposing to tumor formation and the development of resistance to chemotherapy.

Hence, it could be expected that RL, which promotes significant increase in ATP concentration in plasma membranes of tumorous cells [9-12], must adequately enhance the regulatory mechanisms of P53 genes and Bcl family activity.

In our works [9-13] we showed that compounds encoded RI-175, RL-S, and RL-3 accelerate by 60 to 80% the oxidation of succinate by mitochondria of white rats’ liver (1.14 x 10$^6$ c$^{-1}$, against 0.67 x 10$^6$ c$^{-1}$ in control animals) (p < 0.001). ATP levels in the skeletal and heart muscles of Vistar line white rats treated with the preparation under study was 3 to 4 times higher than the control values. Moreover, genuine free energy ($\Delta G$) of ATP hydrolysis in the muscle cells with ATP, ADP, and Pi concentrations of 40, 9.3, and 8.05 mM, correspondingly, pH 7.0, and 25°C, doesn’t exceed $\Delta G$, evolved under ATP hydrolysis in the intact rat erythrocytes, muscles, and liver [21, 22].

$$\Delta G = \Delta G^0 + 2,303 RT \log \left[ \frac{[ADP][P]}{[ATP]} \right] = -12,4 \text{ kcal/mole} (-51,9 \text{ kj/mole}),$$

where: $\Delta G^0$ is standard free energy; R is gas constant; T is absolute temperature; Pi is phosphoric acid.

Then, the difference $\Delta G_1 = \Delta G - \Delta G^0 = -5.1 \text{ kcal/mole} (-21.3 \text{ kj/mole})$ comprises the energy of shift by the investigated compounds of disconnected standard equilibrium constant $K_{eq} = 1,15 \times 10^{-3}[22]$ in the spontaneously connected transformation of A into B in the pool of multi-enzymatic complex of the respiratory chain up to $10^{10}$ times:

$$K_{eq} = \frac{[B][ADP][P]}{[A][ATP]} = 0.28 \times 10^7,$$

where $K_{eq} = \frac{[B]}{[A]} = 5.62 \times 10^3$; ratio $\frac{[ADP][P]}{[ATP]}$ of order 500 [22].

The given calculation testifies to that under the influence of the compounds under study four, and not three ATP molecules, are synthesized in three key segments of the respiratory chain and standard constant equilibrium ($K_{eq}$) in mitochondria shifts $10^{10}$ times, significantly exceeding the similar normal process ($10^{24}$ times) [21, 22].

Along with the known bioenergetic ATP functions (such as movement, active transport, and biosynthetic metabolism), one more function has also been discussed in literature: the amplification of a signal for cell growth and conduction of proliferative stimulus to the nucleus [18, 22]. However, the concentration role of ATP as a mediator of conduction of proliferative stimulus to the
nucleus of a malignant cell, with adequate regulation of gene activity of cell growth factors, has not yet known.

We studied the effect of RL-175, RL-S and RL-175 on the malignant cells of human ovarian carcinoma (CaOy) [11]. The DNA and RNA synthesis rate, as well as protein synthesis were estimated by the introduction of [3H]-thymidine, [3H]-uridine and [3H]-leucine into these cells. A 50% depression of 3HT, 3HU, and 3Hleu inclusion in the tested concentrations 0.5 x 10⁻³ mole was taken as a borderline criterion of the drug activity. The drugs under study almost completely inhibit DNA synthesis and at the same time considerably stimulate RNA synthesis. Thus, under the influence of RL-S in the dose is 10 mcg/ml, the rate of [3H]-uridine inclusion increases by 214% compared to the control value. Correspondingly the protein synthesis increases. These results suggest that RL-like agents contribute to the transfer of the malignant cell through the stage of mitosis (G₂M) to the normal cycle of cell proliferation. In this case, G₂M > G₁S ratio can serve as a regulation criterion of P53 and hence, Bcl-2 family gene activity.

We associate the formation of an excessive RNA pool (matrix, ribosomal, informational, and others) with “edited” extension of the RNA information chain via amplification of that segment of the notional DNA chain, which through genes weakening or disconnecting provoked malignant neoplasms of different location [23, 24].

The concept of “edited” amplification (regulation) of gene expression according to the extension of the notable RNA chain, accounts for indisputable stable recurrence-free remission of malignant focuses (mounting to > or = 50%) in patients with disseminated breast cancer who had demonstrated the failure of up-to-date physicochemical therapy (operation, radiation, hormonal and chemical therapy). We suggest that RL-175 effect on the malignant cells proves the possibility of its regulatory role in P53 gene activity, thus launching apoptosis program and inducing the introduction of malignant cells in the normal proliferation and differentiation cycle. Apoptosis launch is an essential factor in overcoming the resistance barrier of the malignant cells to chemical therapy.

Consequently, the concept discussed here can serve as a serious argument in favor of promising perspectives of RL-175 for prophylaxis of this disease rapidly progressing in the world.

References:
11. Lokhov R.Ye.: Expressomorphogenesis as new direction of biochemical engineering of