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THE PROGNOSTIC MODEL OF ANTITUMOR EFFECT OF TARGETED DRUGS OF IMMUNOTHERAPY

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A prognostic model for constructing hypotheses about the relationship of combinations of cytokines with the proliferative activity of cancer cells is proposed. The model is based on the use of inductive inference methods. The methodology takes into account the synergistic interaction of cytokines and uses sequential construction of logical formulas for selecting groups of cytokines, a statistical analysis of contingency tables and logical integration of the obtained estimates. Implementation of the proposed method in the information system of forecasting the effect of targeted anticancer drugs in immunotherapy will greatly accelerate research in this area.

***Keywords**: information system, contingency table, targeted drugs, immunotherapy, combination of cytokines, cancer cell.*

One of perspective methods of treating oncological diseases is the combination of conventional methods with immunotherapy oriented to the decrease of chemotherapeutics toxicity. Immunotherapy is of particular importance at advanced stages of the disease. In particular, it helps to overcome the resistance of tumor cells to chemotherapy. When chemotherapy or radiation therapy cannot be carried out in view of a serious condition of the patient or a co-morbiditiy, independent application of immunotherapy allows to stop the desease development and to prolong his life keeping a high quality of life [1].

Preparations Refnot and Ingaron developed in Russia showed efficiency for oncological diseases of various location and are being actively implemented in clinical practice [2]. The development of new preparations for immunotherapy including target preparations affecting directly target cells and having minimal influence on healthy cells is at the cutting edge of modern research.

Forecasting antitumoral effect of target preparations for immunotherapy is associated with analyzing the influence of changes in the levels of specific cytokines transcription on the change in proliferative activity of cancer cells [3, 4]. For the pupose of the forecasting it is expedient to use an information system allowing to analyze a large number of interrelating factors. Data on the levels of

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cytokines transcription and the biological activity of lung carcinoma cells were obtained in N.F.Gamaleya Federal Research Centre of Epidemiology and Microbiology.

Biological Experiments

For the cultivation experiments A-549 (human lung carcinoma) cells were chosen from the collection of tissue cultures of N.F.Gamaleya Federal Research Centre of Epidemiology and Microbiology. Conventional medium Igla MEM-90% with 10% fetal calf serum was used for the cells cultivation.

In order to study the cytotoxic effect of the preparations on the cell culture the MTT assay was used. The mechanism and the reasons of the change in the functional cellular activity were determined by studying the production of cytokines at the level of their transcription *in vitro* in cellular cultures after incubation of the cells with the preparations for 24 and 48 h. The gene expression of interleukins IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, of tumor necrosis factor FNO-α, interferones IFN-α, IFN-β, IFN-γ, IFN-λ1, IFN-λ2, IFN-λ3 was estimated taking into account the activity of their mRNA. The activity of the cytokines mRNA in the cells was determined with the use of the reverse transcription and polimerase chain reaction methods [5].

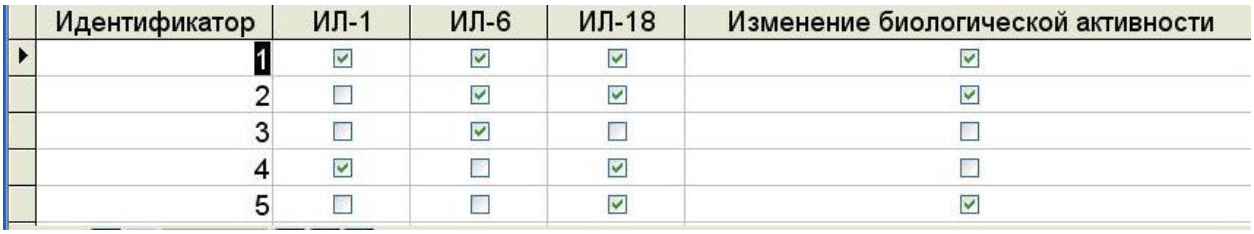
Changes of the cytokine transcription level were estimated qualitatively: it was only found out whether transcription is observed or not. The change of the proliferative activity of the lung carcinoma cancer cells was also estimated qualitatively: whether there are changes as compared to the cheque sample.

Methods, Results and Discussion

Note that numerous studies proved the synergism of the action of cytokines on cells [6]. Many immune reactions are caused by coordinated action of several cytokines. Some cytokines are capable of increasing or decreasing the production of other cytokines. Therefore, when analyzing the influence of the levels of cytokine transcription on the proliferative activity of cancer cells, one should consider the synergetic interaction of cytokines. Thus, the first stage of the study is selection of groups of cytokines, the influence of which should be analyzed in common. A number of such combinations was found and studied in the course of research on the mechanisms of cytokines interaction with cells. Other combinations can be created as hypotheses when analyzing experimental data.

Because all the studied values are estimated qualitatively, they can be considered to be logical variables that can take one of the values “true” of “false”. For the analysis of the interrelation of such variables it is convenient to use the methods of inductive inference. In particular, proliferation change can be represented as a logical function depending on variables describing changes in cytokines transcription level. Essentially, basic data can be considered as the

truth table of such function. It is known that any logical function can be represented by means of operations of classical logic of statements (conjunction, disjunction and denial) in the form of a disjunctive normal form. Figure 1 shows a fragment of an experimental database and an example of creating a logical formula on its basis.



Идентификатор	ИЛ-1	ИЛ-6	ИЛ-18	Изменение биологической активности
1	☑	☑	☑	☑
2	☐	☑	☑	☑
3	☐	☑	☐	☐
4	☑	☐	☑	☐
5	☐	☐	☑	☑

F = (ИЛ-1&ИЛ-6&ИЛ-18) ∨ (ИЛ-6&ИЛ-18)∨(ИЛ-6)∨(ИЛ-1&ИЛ-18)∨(ИЛ-18)

Figure 1. An example of creating a logical formula according to a table describing changes of the levels of cytokine transcription and the biological activity of cancer cells.
[Идентификатор means Identifier; IL means IL – interleukin; Изменение биологической активности means Change in biological activity]

Analysis of the obtained logical formula makes it possible to reveal combinations of cytokines most promicing for further investigation. However, it should be considered that the classical logic allows determining only “strict” cause-effect relations (event A always results in event B). In contrast, inductive inference implies creation and check of hypotheses of possible cause-effect relations (event A, as a rule, results in event B) [7]. So, there can be a situation when the logical function is many-valued (the function value can be different at the same arguments). However, even in this case it is possible to form terms representing the most perspective combinations of cytokines [8].

Statistical analysis was applied for checking and quantitative analysis of interrelations between cytokine groups and proliferative activity of cancer cells.

Cross tables were used as a basic object of statistical analysis [9]. Cross table rows correspond to the cytokine combination (factor), and columns, to the values of proliferative activity (response) [10]. Let us introduce the following designations:

n_{ij} – cross table elements;

$s_i = \sum_{j=1}^{2} n_{ij}$ – the total number of measurements in which the factor takes *i* value;

$c_j = \sum_{i=1}^{2} n_{ij}$ – the total number of measurements in which the factor takes *j* value;

$N = \sum_{i=1}^{2}\sum_{j=1}^{2} n_{ij}$ – the total number of measurements.

The general view of the cross table is presented in Figure 2.

		Response		
		Yes	**No**	
Factor	**Yes**	n_{11}	n_{12}	s_1
	No	n_{21}	n_{22}	s_2
		c_1	c_2	N

Figure 2. The general view of the cross table.

First of all, it is necessary to perform a statistical test of the existence of activity dependence on each factor. For the test it is required to calculate some statistical criteria: Pearson's criterion χ^2, Pearson's criterion χ^2 with Yates's correction, Wald's likelihood ratio test ($\Lambda\chi^2$) and Fisher's exact test.

If significance value (p) obtained as a result of the calculations is less than 0.05 at the chosen confidence probability 95%, the factor affects the response.

Subsequently statistical coefficients estimating the measure of association between each factor and activity were calculated: Cramer's coefficient, Pearson's contingency coefficient, Yule's coefficient of association, coefficient of association by dominance relations.

In addition to the estimation of factor-activity association it is necessary to calculate two coefficients widespread in medical studies:

- relative risk $RR = \frac{n_{11}}{n_{21}} \frac{(n_{21} + n_{22})}{(n_{11} + n_{12})}$,
- odds ratio $OR = \frac{n_{11}}{n_{21}} \frac{n_{22}}{n_{12}}$.

By way of example let us consider the results of statistical calculations for 78 experiments carried out in N.F.Gamaleya Federal Research Centre of Epidemiology and Microbiology. For initial research we chose 6 factors – combinations of cytokines (Figure 3) from publications on lung cancer cytokinotherapy regardless of cancer cells A-549 [11, 12].

Factors	Logical formulas
1	(INFγ&IL-4) & ((¬IL-1) ∨ (IL-6))
2	(INFγ & INFα) & ((¬IL-1) ∨ (IL-12))
3	(INFλ ∨ INFα) & ((¬IL-1) & (IL-2)
4	(INFγ ∨ IL-12) & ((¬IL-10) ∨ (IL-8))
5	((IL-8) ∨ (IL-6)) & ((IL-10) ∨ (IL-4))
6	((IL-4) & (IL-6) & ((IL-2)& INFα)

Figure 3. Cytokine combinations for statistical calculations.

Results of calculations with the use of contingency tables for factor 1 are presented in Figure 4.

		Response		
		yes	**no**	**row sum**
Factor	**yes**	28	14	**42**
	no	14	22	**36**
	column sum	**42**	**36**	***78***

Caluculated coefficients			
Chi-squared test	**X^2=**	6,019	**Significant!**
Likelihood test	**Y^2=**	6,088	**Significant!**
Yates's correction	**JX^2=**	4,953	**Significant!**
If correlated and p= 0,05, values are more than 3,841			
Fisher's exact test	**Fet=**	0,009	**Significant!**
If correlated, value is less than 0,05			
Cramer's coefficient	**v=**	0,278	***Medium assosiation***
Pearson's contingency coefficient	**c=**	0,268	***Medium assosiation***
Yule's coefficient of association	**Q=**	0,517	***Zero if not correlated***
Lower boundary		0,177	Positive value
Upper boundary		0,857	
Coefficient of association by dominance relations			
	Ln(Psi)=	1,115	***Zero if not correlated***
Relative risk	**RR=**	1,714	
Relative risk logarithm	**Ln(RR)=**	0,539	***Zero if not correlated***
Lower boundary		0,077	Positive value
Upper boundary		1,001	
Odds ratio	**OR=**	3,143	
Odds ratio logarithm	**Ln(RR)=**	1,145	***Zero if not correlated***
Lower boundary		0,217	Positive value
Upper boundary		2,073	

Figure 4. Calculation of statistical values for factor 1.

Results of calculations for all the factors are presented in Table 1.

Table 1. Results of calculation of statistical values

Factor number	Criterion X^2	Likelihood test	Yates's criterion	Fisher's exact test	Cramer's coefficient	Pearson's coefficient	Yule's coefficient	Dominance coefficient	Relative risk RR	Odds ratio OR	Factor significance
1	6.01	6.09	4.96	10^{-3}	0.28	0.27	0.52	1.12	1.72	3.14	significant
2	7.12	6.33	5.81	0.008	0.21	0.20	0.53	3.21	2.25	3.23	significant
3	8.3	8.07	7.25	0.003	0.22	0.27	0.48	2.84	2.21	2.88	significant
4	4.64	4.19	3.84	0.022	0.17	0.16	0.45	2.63	1.97	2.62	significant
5	3.42	3.048	2.39	0.046	0.143	0.141	0.445	2.63	1.94	2.60	significant
6	0.71	0.68	0.38	0.12	0.065	0.065	0.18	1.46	1.31	1.43	not significant

It can be seen from the table that the last combination (factor 6) turned out to be statistically not significant for the proliferation of A-549 cells.

Of course, these results should be confirmed and updated when new experimental data are obtained.

The contingency tables used for the statistical unifactor analysis can be used to consider the cooperative effect of the chosen factors. Let us apply the technique of non-uniform sequential procedure [13] to such multifactorial analysis. This technique is based on Wald's method of sequential statistical analysis and allows creating a prognostic scale by calculating special prognostic coefficients and estimating their informativeness.

Development of a prognostic scale by this technique consists of several stages.

First, prognostic coefficients (PC) are calculated for each significant factor. Prognostic coefficients for each of significant factors are calculated according to the following formulas:

$PC(+) = 3 \cdot \ln\left(\frac{n_{11}/c_1}{n_{12}/c_2}\right)$ for the first column of the congingency table (see Figure 2)

and $PC(-) = 3 \cdot \ln\left(\frac{n_{21}/c_1}{n_{22}/c_2}\right)$ for the second column of the congingency table

The results of calculations according to these formulas are rounded to the whole numbers.

Then the threshold values determining three zones of the prognostic scale are chosen: a zone of poor prognosis (where the probability of proliferative activity of cancer cells is high), a zone with unclear (sometimes it is called doubtful) prognosis (where this probability is equal to 50%) and a zone of good prognosis (where this probability is low).

For this purpose the upper (14) and the lower (–9) thresholds dividing the whole prognostic scale into 3 zones were chosen with the use of nomograms [13] (Figure 5).

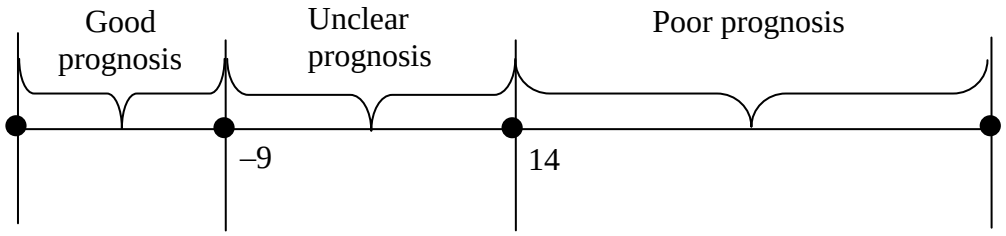


Figure 5. Prognostic scale of the proliferative activity of cancer cells.

Then, when new experimental data are obtained, they are checked for the existence of factors from Table 1. After this, the algebraic sum of the prognostic coefficients is calculated, and it is found out to which prognostic zone it corresponds.

Since the experimental sample is small, it was decided to carry out the assessment of the prognostic properties of the model by the cross-validation method [14]. The mean quadratic error of the prognosis using this method was 23.6%. This error can be reduced by increasing the volume of experimental data and the addition of new significant factors.

Thus, the developed prognositc scale for new experimental data makes it possible to forecast the proliferative activity of cancer cells depending on combinations of cytokines.

The main stages of the technique of forecasting the proliferative activity of cancer cells depending on combinations of cytokines, information required to perform each of the stages and its results are schematically presented in Figure 6.

Conclusions

A model for forecasting the proliferative activity of cancer cells on the basis of hypotheses of interrelation of cytokine combinations is suggested. The technique considers the synergetic interaction of cytokines and uses consecutive creation of logical formulas for the selection of groups of cytokines, the statistical analysis of contingency tables and the creation of a prognostic scale. Realization of the suggested technique as a part of the information system for forecasting the antitumoral effect of target preparations for immunotherapy will allow to accelerate significantly scientific research in this area.

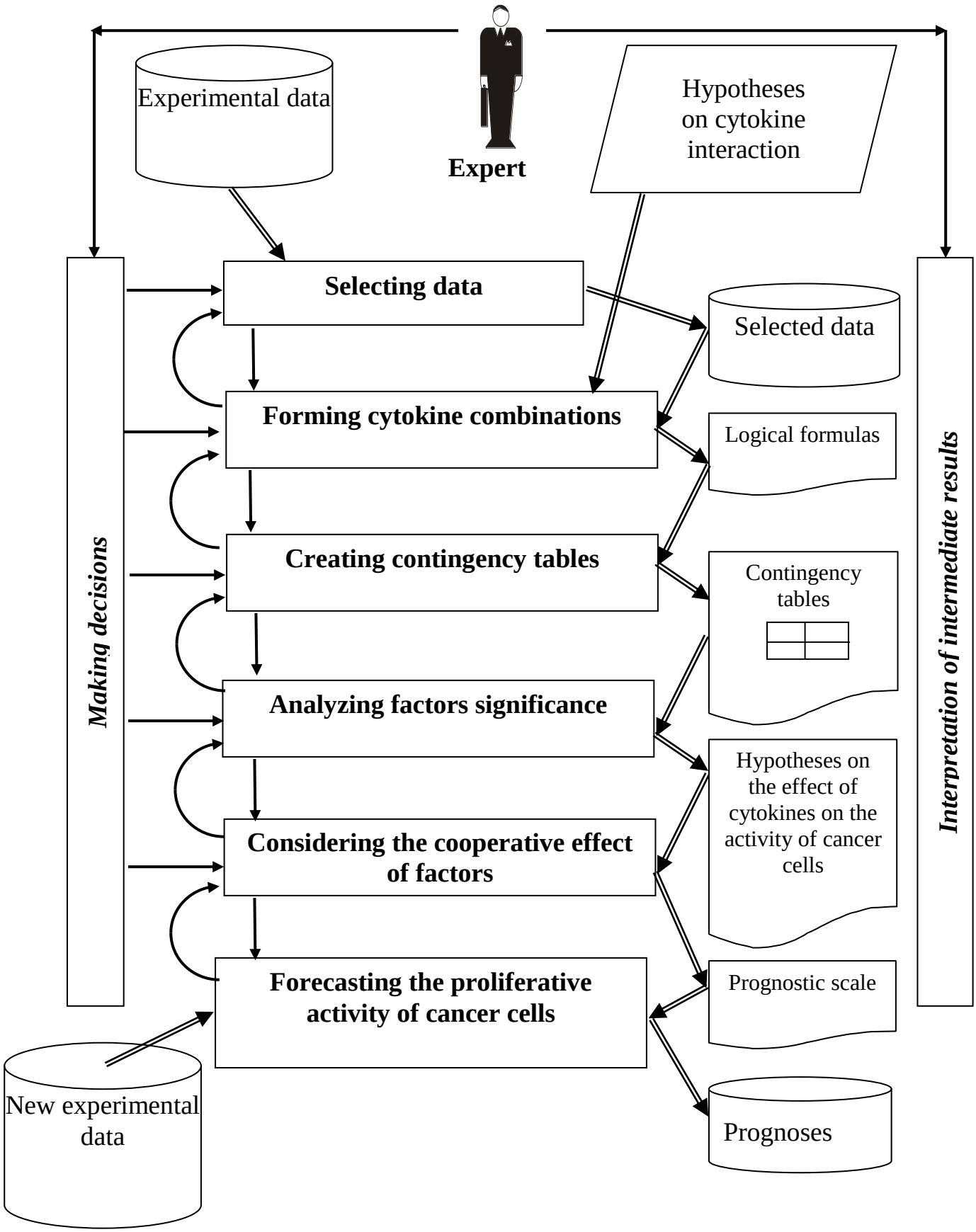


Figure 6. Technique of forecasting the proliferative activity of cancer cells depending on the combinations of cytokines.

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