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A Biokinetic Model to Describe Consequences of Inhibition/Stimulation in *DNA*-Proofreading and -Repair, Part 4

Application of the Model-2

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Summary. In previous papers a strongly simplified physical-mathematical (*biokinetic*) *model* has been presented, which dealt with the factors influencing the timely development of *DNA* mismatches dependent cells (malignant cells) in their kinetic competition to the development of normal somatic cells (*i.e.* cells with correct genetic information). The kinetic results have been studied by comparing them with experimental results reported in the literature upon inhibiting the organism's own enzymatic *DNA*-proofreading and repair machinery. In spite of the fact that the model uses fully the chances of kinetics, which allows to describe even rather complicated systems with many regulation circuits and feed back loops in a rather simple, summarizing way, it has been demonstrated that the model does not only well describe the experimentally found significant increases of mutants in cases when the *DNA* repair system has been inhibited, but it can also reflect cancer-development and the efficacy of classical cancer therapies like *surgery* or *chemotherapy* as well.

In applying the predictions of the model as to the opposite of an inhibition of the *DNA* repair system, *i.e.*, in testing the results, if the organism's own repair systems were stimulated, the model shows that there could be a chance for a *new*, *adjuvant cancer-therapy* if this concept was combined with biochemical facts and clinical findings which are reported in the literature.

In continuation of this concept, the predictions of the model have been compared with findings upon cancer-therapies by *apoptosis-triggerers* like tamoxifen. Further, according to the fact that there exists literature by which it is demonstrated by clinical facts that it is not necessary to use living cells (*e.g.* from umbilical cord's blood or bone-marrow) to achieve surprising therapeutic successes in cancer therapy, but also cell-free "human-placenta-extracts" (*HPE*s) can be similarily effective, it has been tried in a first preliminary analytical effort to characterize "effector-substances" contained therein.

Keywords. Biokinetic model; New adjuvant cancer therapy; Proofreading-stimulation; Equimolar combinations of nucleotides'-precursors; Tamoxifen; Placenta-preparations; *HPE*s; Preliminary analytical screening.

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Introduction

In previous papers a strongly simplified biokinetic model has been presented. Further, it has been demonstrated, that the model reflects well the recently published findings of *Zhang* and *Mathews* [1]: These authors describe the *increase of mutants'-fractions* in cases that *DNA*-proofreading and repair-machinery were inhibited. (See also Refs. [2, 3]). Because the model's output is a description of the timely development of *DNA* mismatches dependent cells (malignant cells) in a kinetic competition to the development of normal somatic cells (cells with correct genetic information), it can also reflect the *efficacy of classical cancer-therapies* like surgery and chemotherapy, by biokinetic modelling.

In a second step, it has been been shown, that also the opposite of *Zhang* and *Mathews*' findings, should be possible: *I.e.*, a *stimulation of the DNA-proofreading and – repair machinery* instead of an inhibition. In an "*if-then-analysis*" by the kinetic model it has been shown that if this is true, this could lead to a new cancer therapy. In a first approach, starting from known biochemical facts [4, 5], the possibility of a stimulation of the organism's own repair-systems by the administration of equimolar combinations of purine- and pyrimidine-nucleotide's precursors (*PUNP*s and *PYNP*s [6]) has been discussed and compared with the model's output. Such a mechanism could further contribute to an understanding of the amazing therapeutic results, which have been achieved by *AICA* [7–13] – a precursor of the purine-nucleotides.

Table 1. List of abbreviations and acronyms used (other than the biochemical standards)

Abbreviation/ acronym	Meaning	Definition
ACG	**A**ctivity of a **C**ancero**g**en (as to carcinogen/cancerogen see also Ref. [2] p. 430 and Ref. [32])	Refs. [2] p. 453 and [3] p. 87 ACG = 0 = no cancerogenity ACG = 1 = "100%" cancerogenity compared to Benzo[a]pyrene
ADPoI	**A**ctivity of a ***DNA*-Po**lymerase **I** analogous system	Ref. [2] p. 445–449; an example for an enzymatic repair-system
AICA	"**A**mino-**I**midazole-**C**arboxy-**A**mide"	Refs. [10, 11] 4-Amino-5-amino-imidazole
BChBkD	**Bioch**emical/**Biok**inetical **D**istance	
C.I.	**C**ancerogenic **I**mpact	Ref. [3] p. 85; the mathematical product of ACG×time of exposure
k_b	**K**inetic "constant" for the formation rate (**b**uilding) of cells	Refs. [2] p. 452 and [3] p. 87
k_d	**K**inetic "constant" for the **d**ying rate of cells	Ref. [2] p. 451
k_{dH}	**K**inetic "constant" for the **d**ying rate of **h**ealthy cells	Refs. [2] and [3] p. 87
k_{dM}	**K**inetic "constant" for the **d**ying rate of **m**alignant cells	Refs. [2, 3]
HPE	**H**uman **P**lacenta **E**xtracts	Refs. [24–28]
OA	**O**rotic **A**cid	

The fact, that it should be possible to stimulate the enzymatic *DNA*-proofreading and -repair machinery has been made likely by the findings of *Kornberg* [4, 5] by the example of the repair-enzyme *DNA*-polymerase I: According to *Kornberg* [4], the enzyme stops its activity immediately upon the lack of only one of the four main-nucleotides (*AMP*, *GMP*, *CMP*, and *TMP*). Therefore it is to be anticipated that usually not all enzymes are at any time completely ''*ammunitioned*'' with these nucleotides. This means that it is likely, that not all of the enzymatic machinery is always active. In reality, just only a part of it will be active locally there, where a *DNA*-lesion has occurred and repair is required. Furthermore, due to the fact that the concentrations in the natural nucleotides pool are relatively low, *i.e.* approximately 10–60 μM [1], such concentrations are rather soon consumed as repair activity is triggered. This effect of a rather soon drop towards zero of the local concentrations in the nucleotides pool where the enzymatic repair-machinery becomes active, is even more stringent the more *DNA*-lesions exist already. This means also, the more cells containing wrong genetic information are already produced and present, the faster the local nucleotides-pool is exhausted. Because of the fact that any subsequent supply of nucleotides from more distant areas is limited mainly by the limits of diffusion-speeds, it will be an option to compensate any lack of nucleotides by offering such by biosynthesis from easily convertible precursors out of depots locally there where they are needed. Thus, it should be possible to stimulate the repair-system.

Because it is known that nucleotides administered as therapeutics are badly accepted in the organism, this option to offer their precursors instead of them must be the preferred method. By this it should also be possible to bypass the biological regulation-systems which control the concentrations in the natural nucleotides-pool. This bypassing might be better illustrated by the expression ''*undertunneling*''. In any way, it would allow to offer the lacking nulceotides to the enzymatic repair-system whenever and wherever they could contribute to a stimulation: To make them available *via* just only a few biochemical steps of converting well selected precursors of said nucleotides. To this purpose, as a therapeutic measure, depots of such precursors could be established at as many positions as possible in the organism.

In addition to *Kornberg*'s principal findings, it has been demonstrated by *Ackermann* [24–28; see also Table 2] that amazing successes in cancer-therapy are achievable by the administration of substances which are neither chemotherapeutics (*i.e*. malignotoxic substances), nor preparations which contain living cells (like stem-cells or umbilical cord cells or bone marrow cells), but which are simple ''cell-free human placenta-extracts'' (*HPE*s), or as they are also called by *Ackermann* ''low molecular'' placenta-preparations''.

In view of (1) this background and (2) taking into account the results which the model delivers in its *if-then-analysis* for the case that a stimulation of the *DNA*-proofreading and -repair system was possible, -plus (3) the therapeutic successes with nucleotides-precursors mentioned above [7–13], it seems highly interesting to consider the possibility of a cancer therapy by effector-substances to stimulate the organism's own repair machinery. For this purpose two options have been pursued:

I) To simulate such therapeutic effects by computing the model's output for increased ADPoI-values by the administration of nucleotides' precursors and by the application of combination – therapies as well, and

Table 2. Case-studies as reported by *G. Ackermann* (1995) [12] according to his ''Immunostimulation/*HPE*''-Therapy (In each case: Histolog. verified Neoplasts at the beginning* (see below column 5) of the *HPE*-therapy; *G. Ackermann* demonstrated the efficacy of his therapy over a period of observation of 17 years with well documented case-histories of each patient during the time of 1956–1970)

Case	Patient	Diagnosis at patient's age/a	1st Therapies	*HPE*-Therapy Start at – till (patient's age/a)	(at the time when published by Ackermann)	
					Survival time[b]/a	Diagnosis
1	Anna M.	Ca. solidum 43	Uterusexstirp. (II. Univ. Frauenklinik Wien)	45*	16	No metastases, no rezidives
2	Maria B.	aplast. anaemia 32 Lymphogranulom 34	*N*-Lost (med. Univ. Klinik Graz)	35*–38 47*–49	15	negative (''o.B.'')
3	Magdalena F.	Ca. mamm. sin. 71	Surg./Radiotherap.[a]	77*	19	no metastases no rezidives
4	Luise G.	Ca. mamm. sin. 68	–	68*–80	12	no metastases no rezidives
5	Maria S.	Scirrh. mamm. sin. 64	–	64*	13	no metastases no rezidives
6	Hermine Ch.	Polymorph. mamm. sin. 62	Surg./Radiotherap.	62*	12	no metastases no rezidives
7	Tere deS.	Neo. mamm. bil. 43/44	Surg./Radiotherap.	46*	9	no metastases no rezidives
8	Anna Sch.	Ca. uteri (corp.) 52	Surg./Radiotherap.	54*	17	no metastases no rezidives
9	Anna M.	Ca. uteri 43	Surg./Radiotherap.	45*	14	no metastases no rezidives
10	Magdalena P.	Ca. vulvae inc. 50	Surg./Radiotherap.	52*	14	no metastases no rezidives
11	Arthur H.	polymorph. Ca. 57 bronch. dx.	Surg./Radiotherap.	57*	13	no metastases no rezidives
12	Gottfried K.	Neo. bronch. dx. 51	Surg./Radiotherap.	51*–60	9	no metastases no rezidives
13	Frieda Sch.	Ca. coli 65 Ca. solid. mamm. sin.	Surg./Radiotherap.	65*–78	13	no metastases no rezidives
14	Edeltraud S.	Osteoidsarkoma 6 dx. antrum	–	6*	18	no metastases no rezidives
15	Roman Sch.	Glioma sin. 6 Gliomrezidiv 6	Surg./Radiotherap.	6*	16	no metastases no rezidives
16	Hans St.	Ca. scrut. chroial. dx. 33	Surg./Radiotherap.	36*	14	no metastases no rezidives
17	Grete K.	Melanosark. eye sin. 48	Surg./Radiotherap.	48*	14	no metastases no rezidives
18	Norman S.	Adeno-Ca. coli rezidiv 47	Surg./Radiotherap.	49*	9	no metastases

[a] Surg./Radiotherap. means surgical tumor-resection, in some cases followed by radiotherapy

[b] Survival times since first diagnosis. According to Ackermann (*loc. cit.*) *i.e.*: ''Survival times immunized neopl. without metastases or rezidives (autoptic confirmed)''

II) to make a first attempt for a preliminary analytical characterization of such potential effector substances.

In another – a third (III) – but conjuncted application of the model, its output for an increase of the k_d-values contained therein was also studied: Because this measure would simulate and reflect the triggering of *apoptosis.* The results given by the model were compared to the benefitial therapeutic effects which offer apoptosis-triggerers (like *tamoxifen*, Refs. [14–16]). With this example the model contributes also to a better understanding of the apparently contradicting results reported in the literature as to the efficacy of this drug [17–23] (see Case 2 and Figs. 2 and 2a).

While pursueing the track of the potential effector-substances to stimulate the repair-system, it must be kept in mind, that the substances as they were isolated by *Ackermann* seem to be those – or their degradation-products, which are present in embryonal systems, where such compounds should be present to do the job necessary, to reduce the risk of formation of erroneous *DNA*-copies in such ''*high-replication-rates*'' systems. Therefore therapeutics made from such embryonal systems – similar to those which have already attracted interest, as there are the placenta and the umbilical cord – must seem promising.

Ackermann's *HPE*-therapy seems to aim towards a similar way as do the modern ''*stem-cells-therapies*'' (german: ''*Stammzellen*'') – or the ''*placenta-blood-therapies*''. However, the *HPE*s must be well distinguished from both of them, while the *HPE*s offer a significant advantage: *Ackermann*'s therapy does not need living cells of very limited availability or long time conserved stem-cells at all, because the *HPE*-therapy seems to be well based upon the stimulation of an enzymatic machinery by well available and well storeable substances.

Biophysical Consequences of Combinations of Therapies

The usual case of a cancer-therapy will be undoubtly a surgical extraction (if possible) of the tumor, followed by an ''as-much-as-possible-damaging'' of any residual malignant cells or metastases by radiotherapy or by chemotherapy.

However, as worked-out in [2] and [3] a good start-ratio, *i.e.*, a low one, of malignant to normal genetic information cells is also essential for any success of an attached stimulation-therapy of the repair-system. A ''*PUNP-PYNP*-therapy'', as it has been suggested according to the already mentioned findings with *AICA* and based upon biophysically/biokinetically reasons, could therefore be of interest and promises success in such cases.

However, even where a stimulation-therapy of the repair-system seems advisable, such a therapy should be employed as an additional (an adjuvant) cancer therapy. It can be classified as a *3rd-generation-therapy.*

The indication ''3rd generation'' should highlight, that such a new therapy would not attack primarily the symptom of the disease, *i.e.*, the tumor, as this is done by methods like chemo- or by radiotherapy, nor would it attack the proliferation-mechanisms (*2nd-generation-therapies*) of tumors, as this is done for example by *angiogenesis-suppressors* as suggested by *Kohn et al.* [29–31]. In this nomenclature, the primarily tumor-attacking therapies like chemo- and radiotherapy should therefore be distinguished by naming them 1st-generation therapies. In contrast to this, a

disadvantage of so called 1st- and 2nd-generation therapies is, that by their character of being mainly tumor-addressing only, they are not focused on mending the source of the disease, *i.e*., the genetic defect, which leads cells to cease their normal behaviour.

Particularly, a therapy to stimulate the repair-system might be reasonable in (a) cases of inoperable cancers like leukaemia, (b) for a cancer-prophylaxis, or (c) as a therapy for very early states of a cancer (''Cancer *in statu nascendi*''). Further, such a *3rd-generation-therapy* may also be of special interest in all cases where the limits of radiotherapy and chemotherapy need to be taken into consideration and a chance remains even in cases of further developped cancers, due to the specialization of the different repair-enzymes of the DNA-polymerase-I-complex (or similars to it) to ''repair'' even *nucleotides*'-*sequences* along *DNA*-lesions (especially *substantial* ones according to Ref. [2]) following ''their *standard-programmes*'': *I.e.* to substitute them against standard-sequences for which they have preferences, – if their activity is stimulated enough – and even if the correct template is not any more available, and the ''repair'' leads to another than the original sequence, – however one, which is not cancerogenic any more. And last but not least, an (auto-) stimulation of the organism's own repair-system – as seldom as it might occur and as extraordinary events might be necessary to trigger it – might also be the reason for the so called ''*spontaneous tumor-remissions*'' which are sometimes observed with cancer. However, spontaneous remissions undoubtly do occur.

Adjustment for Different Types of Cancer

So far, the examplary output of the model has been calculated on the basis of a ''type-1 cancer'' according to the definition *via* the cells' half-life-times given by Ref. [2] (see there: Eq. (3c)). Here it should be demonstrated, that the model can also handle different types of cancers. *E.g*. by adjustment of the cancer-type/cancer-aggressivity parameter to higher values the model shows, that therapeutic measures which led to success with the ''type-1 cancer'' fail at cancers of higher aggressivity. Consequently, a success might only be achieved by adopting stronger therapeutic measures, which means in the case of the administration of stimulators for the body-own repair-system, (a) an administration of higher doses of the *PUNP*s + *PYNP*s, or (b) administration of another corresponding couple of precursors, but those which are selected according to a better adjusted *Biochemical-Biokinetical-Distance* (BChBkD)[a], or (c) using combination-therapies. However, it

[a] **BChBkD** . . . . . **B**io**ch**emical-**B**io**k**inetical-**D**istance.

A nomenclature by which a *PYNP*-n (say: ''*PYNP* minus n'') should indicate a PYrimidine-Nucleotide-Precursor being ''n biokinetic *significant steps backwards*''. ''*Steps*'' being defined therein as: Chemical substitutions or ring-closures or changes in the ring-structure (*e.g*. the dehydration-step from dihydroorotic-acid to *OA*). ''*Backwards*'' meaning that so many of such steps are necessary in the biochemical pathway to form one of the two nucleotides (*CMP* or *TMP*) by conversion of the precursor. Analogously, a *PUNP*-m (''PUNP minus m'') is a precursor which is m of such biokinetic significant steps backwards in the biochemical pathway to form one of the two purine-nucleotides (*AMP* or *GMP*). ''*Biokinetic significant*'' should mean those steps which are not so fast that the variation of the very concentrations of the intermediates involved (like unstable ''transition-states''), do not influence significantly the timely developments of the concentrations of the stable products. This nomenclature is to indicate also other precursor-combinations than *AICA* and *OA* as therapeutica, which might be of different or better depot-efficacy.

turns out that the model is fair enough to demonstrate also the limits of the method, *i.e.*, when a *PUNP* + *PYNP*-therapy would no longer have any chance for being successful. This might occur in those cases, when any dose of such stimulators would be insufficient to stimulate enough the activity of the repair-system. In such cases, the effect of the stimulation will already be limited by the inhibition-effects as reflected by the *pseudo-Michaelis-Menten*-behaviour (see Ref. [3]: Fig. 1), before the kinetic competition might be won. Consequently, the model shows that in such cases a stimulation-therapy alone was not sufficiently effective.

Results

Simulating a "Repair-Stimulation Therapy" and/or the Triggering of Apoptosis

In analogy, respectively in contrast to the effects of the well known *cancer-promotors*, of which efficacy is reflected by the model by increasing the k_b-values, an opposite effect may be triggered by factors which increase the cells'-death-rate. Such factors are known as stimulators of *apoptosis* (see: *Kerr et al.*, *Bursch et al.*, and *Török* [14–16]. By the model apoptosis can be simulated by increasing the k_d-values.

As to the basic mechanisms of apoptosis: It is known that the growth-(rate) of prostata- and mamma-carcinoms are to some extent dependent from the presence of so called "*trophic hormones*" testosterone and "estrogene", respectively, according to *Török* [16]. It is further known that a regression of a hormone-dependent tumor can be triggered by ablation of the corresponding hormone. A similar effect may be reached according to *Bursch et al.* [15] by the administration of hormone-antagonists, *e.g.* tamoxifen.

Tamoxifen is a natural tertiary amine, *i.e.* chemically a substituted dimethyl-ethylamine of the type *N*,*N*-dimethyl-2-[4-(1,2-diphenyl-1-butenyl)phenyl-oxy]ethylamine (see: *Römpp* [32]) and it acts as an antagonist to "estrogene".

The effect of such administrations, simulated by the model, may be demonstrated by the following examples:

Case 1: No Therapy versus "Stimulation-Therapy"

Using the computer-program CANCER.xls with an input of a long lasting, rather serious cancerogenic impact (C.I.). (C.I. = 20 periods; time of observation = 1000 weeks, *i.e.*, 20 periods × time per period: 50 weeks; ACG = 30%; for a "type-2 cancer" (*e.g.* a mammatumor)). The calculation delivers several results:

Result No. 1-1: An unstimulated *DNA*-repair-system does not allow the patient to survive longer than 150 weeks (= below 3 years).

Result No. 1-2: A *stimulation of the DNA-repair-system to 30-times of its normal activity* (*i.e.* to $\mathrm{ADPoI} = 30 \times 10^{-6}$ by a daily *PUNP* + *PYNP*-administration of 1.2 mg/kg b.w. (see Ref. [3], there Table 2 = approx. 70 mg/d of the stimulator)) changes the development to a reduction of the number of malignant cells with a flattening-out of the fallbacks (Fig. 1).

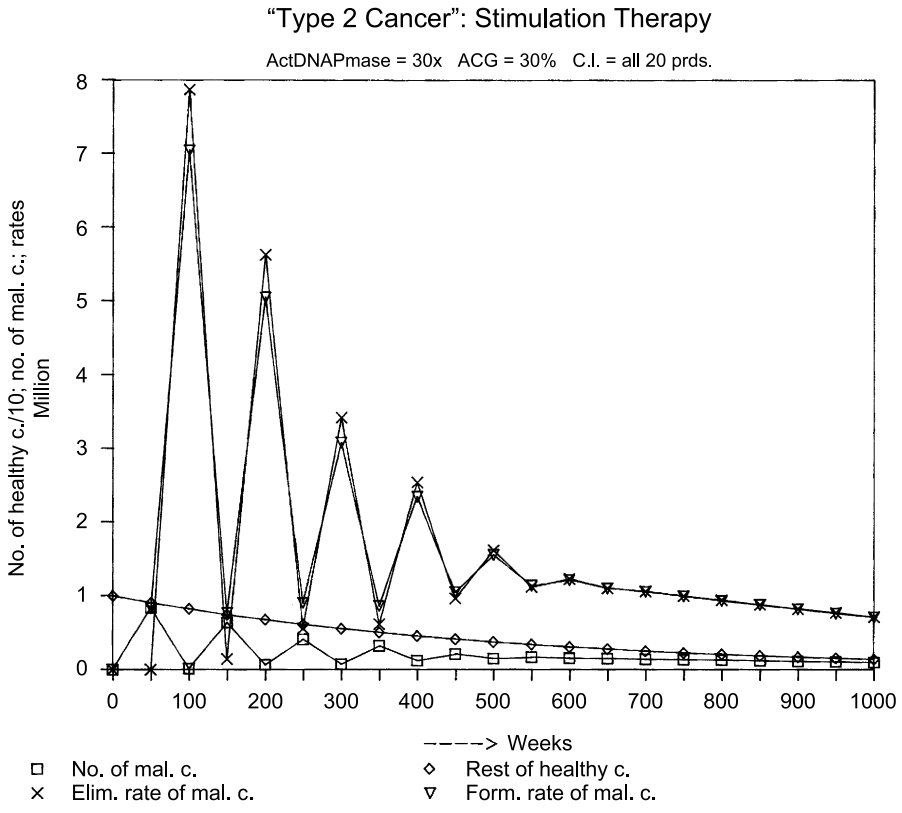

Fig. 1. A "type 2 cancer" (see Ref. [4] *e.g.* a Mammatumor); repair-system stimulation therapy by the administration of effector-substances to stimulate the organism-own repair system to 30-times of its normal activity (healthy-cells scaled to $1/10$)

Result No. 1-3: In contrast to this: Just only a stimulation to $2/3$ of the former case would fail. (*I.e.* $\mathrm{ADPoI} = 2\times10^{-5}$ only, by only 40 mg/d, *i.e.* approx. 0.7 mg/kg b.w. of the stimulator, instead of the above mentioned 1.2 mg/kg b.w., Fig. 1a).

Case 2: Simulating a Therapy with an Apoptosis-Triggerer (e.g. Tamoxifen)

Simply by increasing the value of k_{d}. (As a further "complication", such a therapy would obviously stronger influence k_{dM} rather than k_{dH}. But even such an effect could easily be handled by the model, because it could mathematically be also described by keeping a definite ratio $k_{\mathrm{dM}}/k_{\mathrm{dH}} > 1$. And this condition could be simplified in the calculation by increasing the value of k_{dM} only). The model delivers for this case:

Result No. 2-1: Continuing from the last example: As an additional measure to the not sufficient repair-system stimulation-therapy as applied in case 1–3 above, also the k_{dM}-value is increased from its default-value of 6 to 7. (Meaning an increase by just only 15% as it might reflect the triggering of apoptosis by the

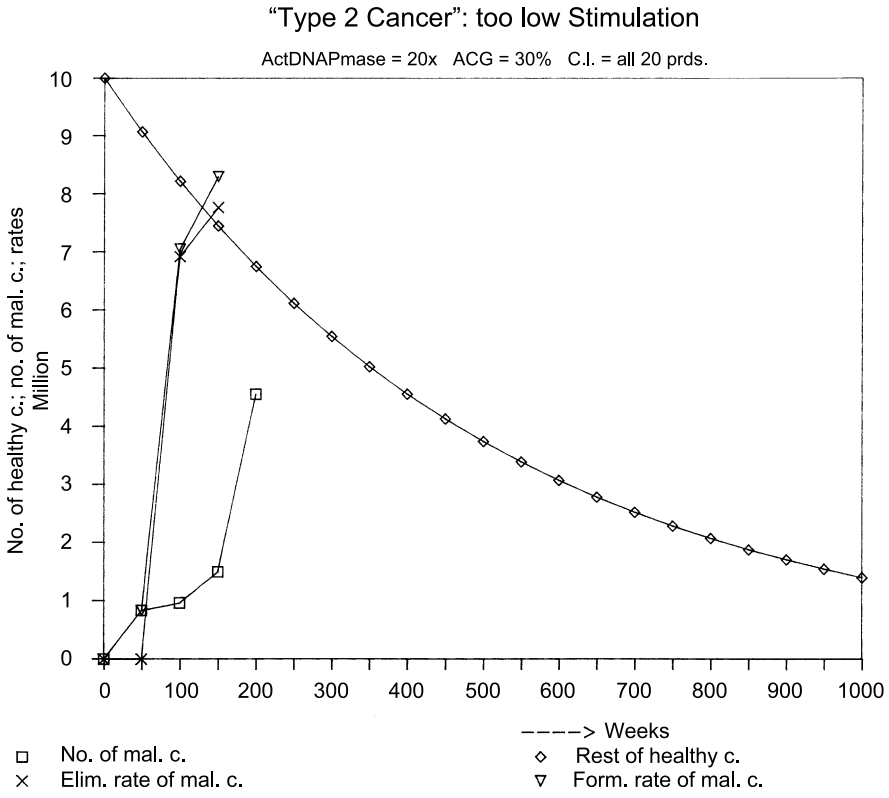

Fig. 1a. Same as in Fig. 1, but repair-system is stimulated only to 20 times of its normal activity

administration of tamoxifen). The computed result shows, that already the lower dose of *PUNP* + *PYNP*, which led the therapy to fail in case 1–3, becomes now sufficient (Fig. 2). This example indicates how a combination-therapy of a stimulation of the repair-system together with a hormone-antagonist-administration could work.

Further, a pure apoptosis-therapy with omitting the stimulation of the repair-system can also be simulated by the model:

Result No. 2-2: Increasing k_{dM} up to 9. (*I.e.* by +50%, which corresponds to a very significant increase in malignant-cells'-death-rate by apoptosis. For such an extreme case it is to consider if such high increase can be possible at all just only by the administration of a hormone-antagonist). However, the calculation demonstrates, that in such a case, even with an unstimulated *DNA*-repair-system, a similar therapeutic success might be achieved at least theoretically (Fig. 2a). Nevertheless the model shows that it were better to use a combination-therapy as shown in case 2-1 above.

By these biophysical/kinetic explanations of the therapeutic effect, which tamoxifen may have, it might also be possible to deliver an explanation, why recent studies of a British researchers-group led by *Powles et al.* [17] and of an Italian researchers-group led by *Veronesi et al.* [18] showed that tamoxifen was inactive in

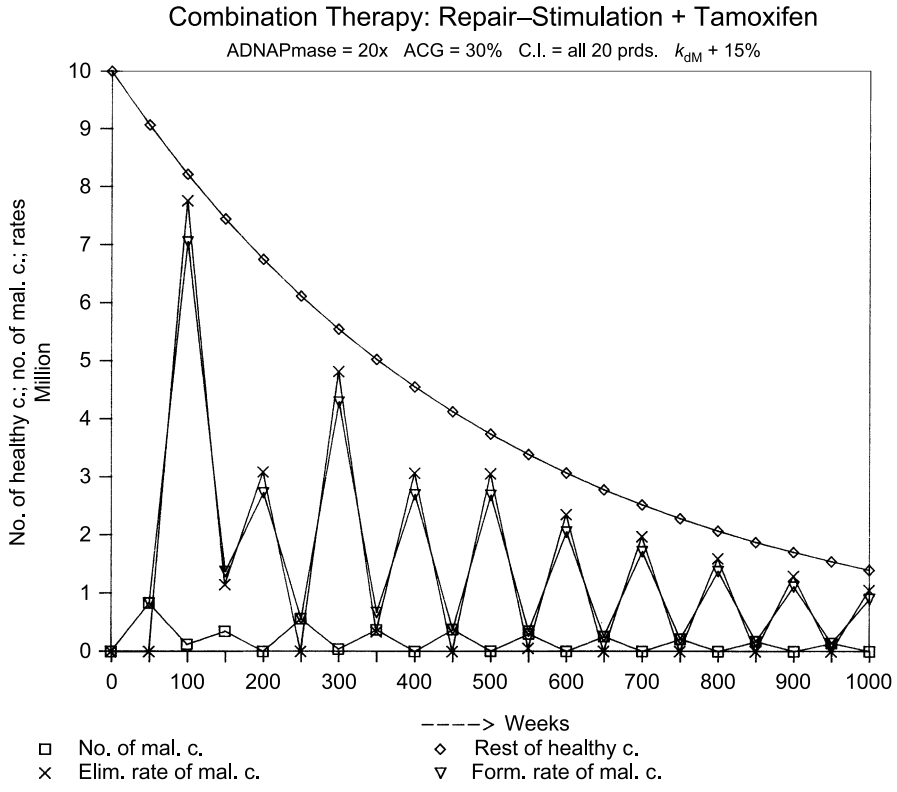

Fig. 2. Same as in Fig. 1a, but also apoptosis is stimulated by tamoxifen (simulated by k_{dM} increase by 15% (from 6 to 7))

the prophylaxis of mammatumors. While in contrast to this, tamoxifen was found to be effective according to American studies on mammatumor "high-risk" patients carried out by *Jordan* [19], by the Early Breast-Cancer Trialist's Collaborative Group [20], by the Novaldex Adjuvant Trial Organisation [21], by *Cuzick & Baum* [22], and by the NCI [23].

Basic facts: The "*Powles*-study" included more younger women: 62% aged under 50. Also the "*Veronesi*-study" was focused more on "low(er)-risk women": Only 18% with one first-degree-relative aged under 50 with breast-cancer, respectively only 2.5% with two or more first-degree-relatives of any age with breast cancer. In contrast to these, the "*Cuzick & Baum*-study" was more focused on "higher-risk women" because in this test-group there were only 40% aged under 50, but 55% with one first-degree-relative aged under 50 with breast-cancer, respectively 17% with two or more first-degree-relatives of any age with breast cancer.

Biophysical/biokinetic explanation: It might well be that with the younger and "low-risk women" in the British and Italian studies there were not any much malignant cells, or malignant genetic information precursing them, present in the statistical average to obtain a significant shift of the kinetically determinating ratio of healthy cells to malignant cells by apoptosis. This effect is demonstrated in the version CANCERt.xls of the program offered.

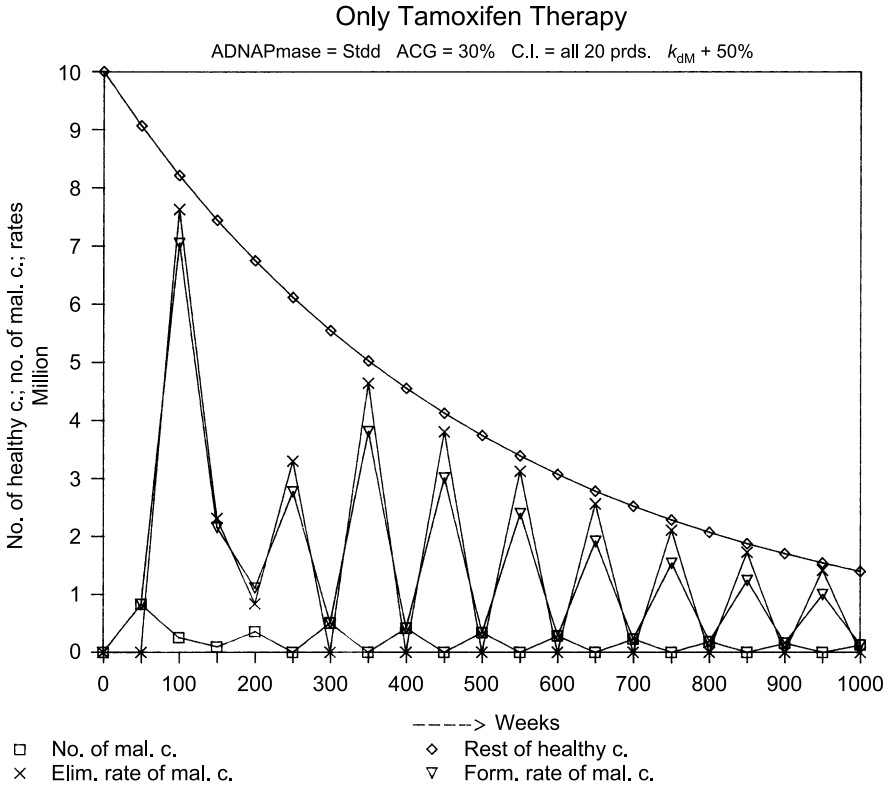

Fig. 2a. Same as in Fig. 1, but apoptosis is stimulated by tamoxifen only (simulated by k_{dM} increase by 50% (from 6 to 9), no additional stimulation of the repair-system)

Safety Remarks as to Combination-Therapies

In contrast to the positive effects demonstrated above by the model for combining the therapy for the stimulation of the repair-system by nucleotide-precursors with a therapy to trigger apoptosis, *e.g.* by tamoxifen, an immediate combination of a *PUNP-/PYNP*-therapy with toxic, *e.g.* toxic halogen-compounds containing therapeutics, or with cytostatics which contain ''blocker-groups'', *e.g.* such which are chemically similar to L 651 582 or fludarabine, cladribine, or pentostatine, might be dangerous. At least such combinations could act inter-deactivating, due to affecting the same enzymatic systems but in antagonistic ways.

Reports on Experimental Evidences for Successful Cancer Therapies – Possibly Related to a PUNP + PYNP-Therapy

Apart from the chances which offer pure combinations of *PUNP*s and *PYNP*s as there are 5′-phosphoribosyl-*AICA* or *AICA* itself (as the *PUNP*) and orotic acid (as the *PYNP*), – according to the model given – there should exist also several other compounds, which possibly might give a similar therapeutic effect. Especially those among them should be of interest which exhibit some kind of a timely buffer – or depot-effect. Such effects could be reached by administering precursors which are ''more back'' in the chain of the biochemical pathways, *i.e.*,

precursors with higher biochemical-/biokinetical distance in the biosynthesis to the nucleotides. But also natural products could be of interest, if they contain such substances or are able to release such substances during metabolism.

As mentioned above, milk – especially sheep's milk – is according to *Gordonoff* and *Schneeberger* [33] at least a source for orotic acid and by this for a *PYNP*. Thus milk – or milk-products like curd cheese *etc*. – were such a carrier and could be one component of a *PUNP* + *PYNP*-therapy, if the second component (*i.e*., the *PUNP*-component) was supplied by other sources.

The vitamine-B-complex containing vitamine B_2, the *FAD*s (flavine-*adenine*-dinucleotides), due to their adenine-content might be a natural source for a *PUNP* analogue to *NADH* according to *Birkmeyer* [34].

However, there are also other natural sources, which might also carry similar active substances. Most likely, such substances should be found especially where high replication-rates are executed in an organism. With high replication-rates also the risk for production of erroneous *DNA*-copies is increased. Thus, it is to be anticipated that exactly there mechanisms of higher efficiency in the repair of mismatches or lesions in *DNA* have been created by the evolution. Exploiting this effect might be: After 17 years' experience with a cancer-therapy based upon *i.m*. administrations of ''*low-molecular human placenta extracts*'' (''*HPE*'') impressive successes in stabilization of serious cases of cancer are reported by *Ackermann* [24–28]. However, these successes are well to be distinguished from recently reported successes in therapy of leukaemia with stem-cells (so-called ''Stammzellen-therapies'') from umbilical cord blood. *Ackermann*'s preparation-method damages all living cells by repeated lyophilisation-, freezing-, and centrifugation-processes to isolate substances contained in the cells' cytoplasm; eliminating all living cells and cell-membranes and by this being mainly focused on ''low-molecular'' substances contained in placental blood and the cells therein.

As to nomenclature: Obviously, *Ackermann* meant with ''*low-molecular*'' (german: ''niedrigmolekular'') not necessarily only substances of low molecular weight – *i.e*. excluding polymers and oligomers. *Ackermann*'s german denomination ''niedrigmolekular'' should better be translated by ''free from living cells and free from high-molecular cell-materials like cells' membranes; not necessarily free from oligopeptides, proteins, protoenzymes, or even enzymes''.

Ackermann's data have been supported by serious studies of his *HPE*-therapy on mammatumor-mice. *Ackermann* [25] stated that the mechanism of the *HPE*-therapy is based upon a stimulation of the ''immuno-system''. Therefore *Ackermann* focused his preparation-method in the following years (see Ref. [27]) towards the preparation of ''low molecular'' (say better: cell-free) ''immunisation-sera''. As a result according to these considerations and of the method which *Ackermann* chose for the preparation of the *HPE*s following his interpretations of the therapeutic effect which he found, there arose problems with the protein- or peptide-contents of the first sera. Caused by these, pyrogenity of the *HPE*s was reported. Nevertheless, the therapeutic successes were impressive in at least twenty-seven well documented cases of cancer reported by *Ackermann* [24] (the 18 most convincing are listed in Table 2).

Seen in the light of the model presented, *Ackermann*'s successes might become once again of interest. At least as indicators that further research is promising.

Particularly, because in some cases of the *HPE*-preparations highly interesting *effector-substances* might have been really extracted and conserved. In this respect, the above, surprising therapeutical successes might be well founded in a manner similar to the also surprising findings of *Rubinstein* [35]. He achieved therapeutic successes with cord blood-administrations to patients who were gravely ill with leukemia, lymphoma, other cancers, or genetic diseases. *Rubinstein* was forced to use cord blood as a last resort, because no bone-marrow-donors could have been found in time for his patients. This successful use of placental blood was also similarily commented by *L'Enfant* [35]. Further similarities might be the surprising therapeutical successes of *Birkmeyer* [34] by administrations of *NADH* and/or *NADPH*.

However, a 2nd effect should also be considered, *i.e.*, it could be that *Ackermann*'s *HPE*s did not only contain nucleotide-precursors which acted as stimulators for the *DNA*-repair-machinery, but also some enzyme- *etc*. precursors which acted really as effector-substances stimulating the organism-own system for eliminating also established malignant genetic informations and cells. Such a 2nd mechanism could be for example a stimulation of *tumor-suppressor-functions* as they are introduced in a healthy organism by the expressions of working "*tumor-supressor-genes*".

Remembering that it is a fact that cases of "*spontaneous tumor-remissions*" do occur, irrespectively to how seldom such cases might be recorded and how poor

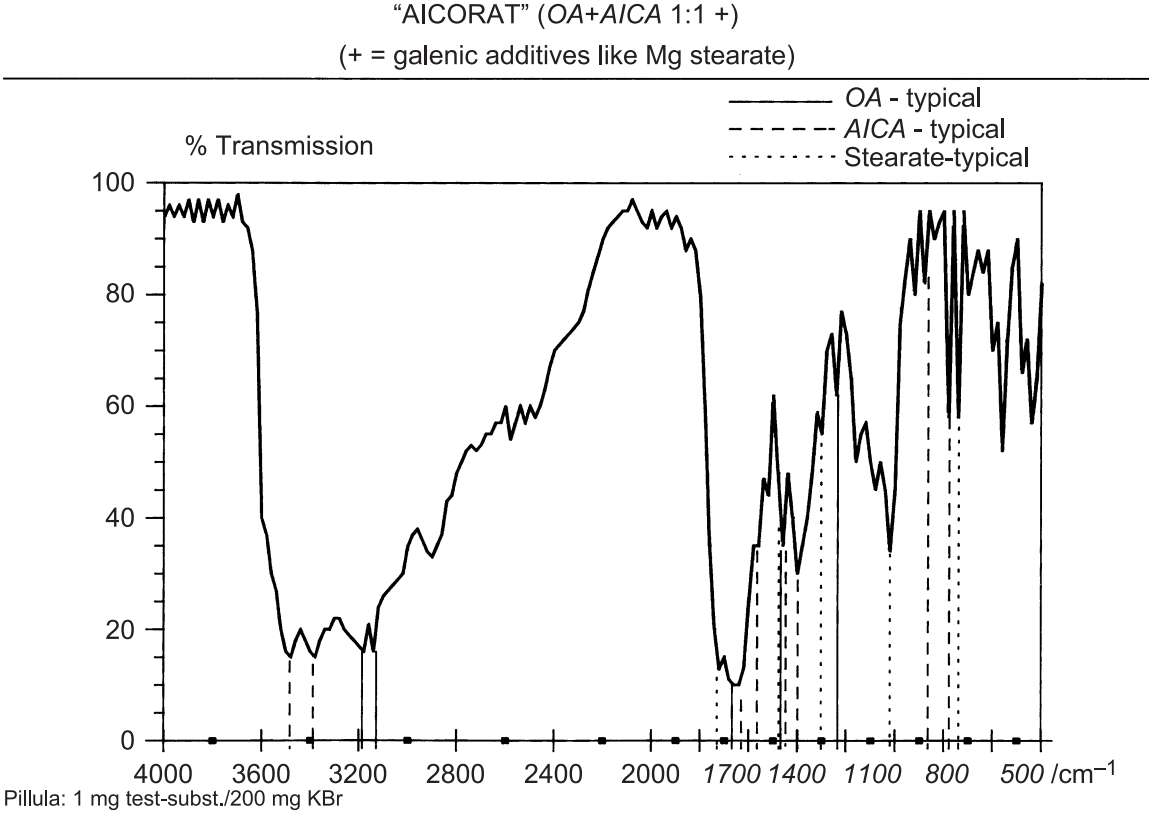

Fig. 3a. IR-spectrum of a galenic formulation of *AICA*-orotate (containing stearic acid) "AICORAT"

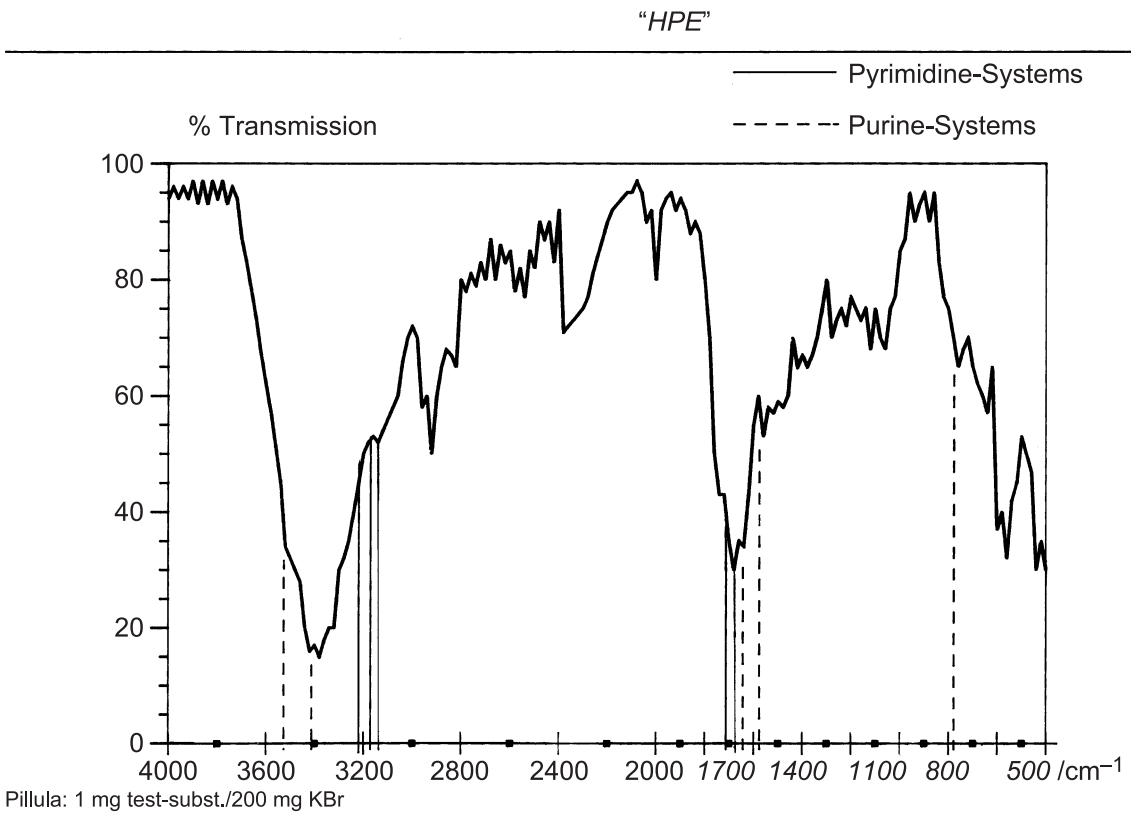

Fig. 3b. IR-spectrum of Ackermann's *HPE*

their scientific understanding is till today, this fact demonstrates that mechanisms must exist in an organism which are able to fight cancer. Thus, it should be of high interest to explore these organism-own mechanisms and how they can be stimulated. *Ackermann* at least has proven that a successful cancer therapy is possible by the administration of specific substances (not cells). Substances which are not malignotoxic as the chemotherapeutics are. Obviously effector-substances which have not been characterized chemically till now.

Preliminary Analytical Tests

While *NADH* and *NADPH* are well known and chemically well defined substances compared to the background described above, it seemed to be of urgent interest to make a first attempt to characterize *Ackermann*'s *HPE*s.

Due to the lack of an appropriate lab-capacity and lab-equipment, till now it was only possible to realize preliminary, first analytical tests. It was the scientific interest in the matter "to have any insight into such substances" which seemed to justify this attempt. The author is aware of the fact that better analytical methods should be employed and also modern separation-methods like HPLC should precede the analytical efforts. Further it is desirable to use hyphenated methods.

Nevertheless – because of the high actuality of modern cancer therapies other than chemotherapy, *i.e.*, therapies which are focused on the administration of substances derived from embryonal environments – it was tried in this first attempt to

submit samples of *HPE* directly to IR spectrometry. The *HPE* samples used had been originally forwarded by *Ackermann* [27]. They had been prepared under standardized conditions according to *Ackermann*'s prescriptions at Serotherapeutisches Institut (today in: A-1230 Vienna).

The spectra have been compared with the spectra of nucleotides, nucleic bases, and the precursors mentioned in this paper as they have been recorded by *Pouchet* [36] and with the results of earlier investigations of *Brownlie* [37], *Short* and *Thompson* [38], *Tanner* [39], *Rao* [40], and *Bellamy* [41], which investigations had been focused very specifically to the IR absorptions of nucleotides, substituted and deuterated pyrimidines and purines.

As a first result: It is obviously well possible to identify the typical absorption bands of *AICA* and of *OA* in the spectrum of the medicament AICORAT (*i.e.*, orazamid $\cdot 2H_2O$ with some stearic acid as a galenic additive). Mainly, the spectrum can be interpreted as a superposition of the typical absorption bands of *AICA* and *OA*.

Accordingly there can also be detected typical similarities of *OA* with other substituted pyrimidine systems (see Tables 4/1–4/4 and 5 and compare Fig. 3a) and of *AICA* with substituted purine systems (see Tables 3/1–3/4 and 5 and compare also Fig. 3a).

Table 3/1. Purine systems; the region of $\bar{\nu} = 3400$–$2800\,\mathrm{cm}^{-1}$, *i.e.* at the very strong C–H and N–H stretching frequencies; represented by *AICA* as the typical *PUNP*, adenine and guanine as the corresponding nucleic bases, and for the purpose of comparison also purine itself as the unsubstituted ring-system, plus uric acid as the fully carbonylated (only ring-NH) type (see also Refs. [36, 40])

Group	Significant IR-bands; $\bar{\nu}/\mathrm{cm}^{-1}$				
	AICA	Adenine	Guanine	Purine	Uric acid
NH_2	**3485** a **3380** s[a]	3300 a 3130 s	3300 3120	–	–
resp. ammonium (in case of hydrochlorides)	(3320)				
OH					3120 (weak; from the only small tautomeric part[b])
ring-NH (corresponding to the ring-H-doublett of the imidazole- resp. purine-type systems)	**3185** **3130** 3060			3080	
ring-CH		2930[c] 2860[c]	2930[c] 2860[c]	2940	(2920)[c]

[a] Taking into account shifts by association and partial ammonium-dissociation, but in any way relative good meeting Bellamy's [41] relation between symmetric and antisymmetric NH vibrations: $\bar{\nu}_s$ (approximately) $= 346 + 0.876 * \bar{\nu}_a$

[b] Indicating that the aromatic-tautomeric-forms are not enough supported energetically in the substituted molecules

[c] The sharp doublet-absorptions at 3.4 and 3.5 microns (*i.e.* approx. 2930 and $2860\,\mathrm{cm}^{-1}$) as well as the absorptions at $\bar{\nu} = 1450$ and $1400\,\mathrm{cm}^{-1}$ are due to sample preparation (Nujol Mull) if the samples are not measured in the form of KBr-pillulae; to some extent they are overlapping with real sample absorptions

Table 3/2. Purine systems; the region of $\bar{\nu}$ = approx. 1700 cm^{-1}, *i.e.* the typical strong carbonyl-bands of the tautomeric forms of the ring-OH (Ref. [39])

Group	Significant IR-bands; $\bar{\nu}/cm^{-1}$				
	AICA	Adenine	Guanine	Purine	Uric acid
C=O	**1685**[d]	–	1720	–	1680[d]
(and ''control-band'')	**1650**[d]	–	1670	–	1670[d]

[d] Compared to normal carbonyl-groups typical shift to lower frequencies of the bands of the ''pseudo-carbonyl''-groups

Table 3/3. Purine systems; the region of ring-N=C and C=C and C=N bands at the so called ''finger-print-region''

Group	Significant IR-bands; $\bar{\nu}/cm^{-1}$				
	AICA	Adenine	Guanine	Purine	Uric acid
ring-N=C		1670		1620	
C=C, C=N	**1570**	1600	1550	1560	1550
	1470	1460	1450	1460	1450
	1400				

Table 3/4. Purine systems; the region of γ- and δ-bands; C–N and C–H bands; ''finger-print-region for ring-systems''

Group	Significant IR-bands; $\bar{\nu}/cm^{-1}$				
	AICA	Adenine	Guanine	Purine	Uric acid
Deformation-frequencies (in-plane-bending?)					
		1310	1350	1270	1300
	1250	1250	1260	1200	higher symmetry → no band
	1150	1120	1120	1100	1120
	1100				
		1020	(1040)		1020
Deformation-frequencies (out-of-plane-bending?)					
	940	940	950	970	990
				925	
	890	910		910	
		870	880		870
		840	840		
	780	790	780	780	780
	750	725	700	720	740

Table 4/1. Pyrimidine systems; the region of $\bar{\nu} = 3400$–$2800\,cm^{-1}$, *i.e.* at the very strong C–H and N–H stretching frequencies; a doublet of the ring-N–H (*i.e.* with the exception of unsubstituted pyrimidine; α CH is observed in this case[e]) indicating that the aromatic-tautomeric-forms are not[f] enough supported energetically in the substituted molecules (see also Refs. [36, 40])

Group	Significant IR-bands; $\bar{\nu}/cm^{-1}$				
	OA	Thymine	Cytosine	Uracil	Pyrimidine
O–H	–	3360			single peak: **aromatic**, *i.e.*, no different ring-N–H
NH_2			3400 a 3170 s		
N–H I	**3150**[f]	3180[f]	see above	3180[f]	
N–H II (stronger)	**3090**[f]	3070[f]	[c]	3090[f]	
C–H** (valence-bands Short and Thompson [1952])				[c]	3040[e]

[c] The sharp doublet-absorptions at 3.4 and 3.5 microns (*i.e.* approx. 2930 and $2860\,cm^{-1}$) as well as the absorptions at $\bar{\nu} = 1450$ and $1400\,cm^{-1}$ again are due to sample preparation (Nujol Mull) if the samples are not measured in the form of KBr-pillulae

Table 4/2. Pyrimidine systems; the region of $\bar{\nu} =$ approx. $1700\,cm^{-1}$, *i.e.* the typical strong carbonyl-bands of the tautomeric forms of the ring-OH (Ref. [39])

Group	Significant IR-bands; $\bar{\nu}/cm^{-1}$				
	OA	Thymine	Cytosine	Uracil	Pyrimidine
C=O	**1690** (1650) Carboxylate in conjugation	1730 1670	1640 broad	1720 1650	–

Table 4/3. Pyrimidine systems; the region of C–H and N–H stretching frequencies-bands at the so called ''finger-print-region''; typical the pyrimidine-ring-triplett

Group	Significant IR-bands; $\bar{\nu}/cm^{-1}$				
	OA	Thymine	Cytosine	Uracil	Pyrimidine
	1490	1490	1530	1510	1560 (Ref. [37])
	1470	1470	1495	1470/50	1470
	1430	1440	1450	1420	1400

While, according to these IR spectra it has been found that *HPE* is not identical with any single nucleotide nor any equimolar mixture thereof, nor of any of the precursors mentioned in this paper (like $AICA + OA$), this result should not be interpreted as being too disappointing. For unfractionated extracts of a natural

Table 4/4. Pyrimidine systems; the region of γ- and δ-bands; deformation-frequencies; in plane-bending

Group	Significant IR-bands; $\bar{\nu}/\mathrm{cm}^{-1}$				
	OA	Thymine	Cytosine	Uracil	Pyrimidine
CH	**1220**	1240	1230	1240	1220
	1110	1200	1150	1100	1160
	1020[g]	1030	1010	1010	1070[g]
(The region of the deformation-frequencies;					
out-of-plane-bending)[g]	**920**	930	960	990	lacking of some bands
	880			850	
		830	820	820	820[g]
	810	810			due to higher symmetry
	760	760	790/780	(780)	
	720	740		760	720

[g] Ref. [39]

product, no other results were to be expected. Just because of the fact that the IR spectra of an 1:1 *AICA* + *OA* mixture are not congruent with those of *HPE*, it is too trivial to put aside any possible similarity between *Ackermann*'s therapeutic successes and the theoretically sound option for a stimulation-therapy of the body-own repair systems.

Nevertheless and indeed, there are some bands and bands-configurations which are known from the literature as far as it is specificly focused on infrared spectrometric analytics of purine and pyrimidine systems and which do indicate similarities. Particularly by concentrating on the regions of such bands-configurations which are characteristic for substituted imidazole and pyrimidine systems, respectively, which are also able to give tautomeric-forms, similarities have been detected. However, it is obvious that only a careful comparison of all IR spectroscopic data as they are typical for the whole group of purine and of pyrimidine derivatives (as given in Tables 3/1–3/4 and 4/1–4/4; compare Table 5 and Fig. 3b) with those of the *HPE*s, as it was done in this first attempt, entitles to a deduction of such ''similarities''. It is as well obvious, that further, more detailed analytical studies are still necessary.

What is intended in this first step is to point out that the results of *Ackermann* and those of recently described cancer-therapies employing substances derived from embryonal environments, together with the ''if-then-results'' of the model presented and the data available till now (as much as these data must be items for further deepening and improvement) should give reasons enough for further research with this kind of potential therapeutics.

Experimental and Method

Spectrograph used: PERKIN ELMER PE 882; Slit resolution: 2.4 cm^{-1}; filter-noise: 0.5%T. The samples to be measured were mixed with KBr and pressed to pillulae insertable into the spectrograph.

Table 5. Typical IR-bands in the spectrum of AICORAT and *HPE*

Group	Significant IR-bands; $\bar{\nu}/\mathrm{cm}^{-1}$			
	Galenic formulation "AICORAT"			*HPE*
	AICA-typical	*OA*-typical	stearate-typical	
NH_2 (from amino- resp. amido-groups)	**3490 a**			**3500** broad peak
	3380 s			**3400** +shoulder (HO- and H_2N-groups?)
ring-NH	**3185**			
	3130 ← coinc. →	**3150**		**3100** sub-shoulder where the ring-N–H of purine and pyrimidine systems do absorb
		3060		
C=O	**1670** ← coinc. →	**1680**	1700	(1700–1720)[h]
				1680[i]
C=N, C=C	**1580**			**1570**
	1470 ← coinc. →	**1470**	1480	**1470**[j]
	1400			
			1320	
Deformation frequencies	1100	**1230**		
				1120 –
		1010		Deformation-frequencies -series similar to purine-
			960	
		920		– 900 nucleic bases (Refs. [39, 40])
	890 ← coinc. →	**880**		
			730	

Similarities

[h] The typical normal carbonyl-absorption at $\bar{\nu} = 1720\,\mathrm{cm}^{-1}$, respectively the carboxyl/carboxylate-absorption in the same region is rather weak, indicating that *HPE* does not contain significant amounts of amino acids.

[i] The "shifted carbonyl-band" at $\bar{\nu} = 1680\,\mathrm{cm}^{-1}$ is much stronger as it is known from the tautomeric, non-aromatic forms of the purine- and pyrimidine-derivatives (Tanner [39])

[j] The peak at $\bar{\nu} = 1470\,\mathrm{cm}^{-1}$ is well positioned and of similar sharpness as the corresponding typical peaks of *AICA* and *OA*

Equimolar Mixture of Orotic Acid and AICA

Tablets of AICORAT (made by HEINRICH MACK NACHF., D-89252 Illertissen, Germany) containing as to producer's data 100 mg of "Orazamid · $2H_2O$" per each dragée in a composition with Mg stearate were peeled-off from their outside coat. The inner white, pressed substance was scratched out and mixed under grinding with KBr (1 mg/200 mg KBr).

Conclusion

By a simple biokinetic model there can be well simulated experimental results as they are described in the literature upon the increase of mutant fractions in cases that the *DNA*-proofreading and -repair-system has been inhibited. As well as these, also clinical results achieved with classical cancer-therapies, or with

apoptosis-triggerers like tamoxifen can be described. There arise further predictions from the model by which interesting chances for a potential new cancer-therapy are indicated: A "*repair-system stimulation-therapy*" should be possible.

Seen from this biokinetic point of view, also successful new cancer-therapies which have been reported recently in the literature (like administrations of *bone-marrow* or of *umbilical cords'-blood*), but also earlier attempts (like therapies with *Ackermann's* "*low-molecular human placenta-preparations*) seem to influence mechanisms which are understandable in a biokinetical similar way.

These results should be interesting enough to suggest further research in the field of the stimulation of repair-systems and they should in any way encourage a partner for such a research program.

Acknowledgements

The author is indebted to *I. Caspar* – being herself *G. Ackermann*'s longest surviving "case" of a cancer-patient – for making available samples of *Ackermann*'s *HPE*s out of her personal medicamental stock for her personal therapy.

Extremely appreciated were also the efforts of *Dr. F. Fuchs* for making possible the measurements by infrared-spectroscopy and his contributions out of his experiences in practical IR-spectrometry. The author has also to thank this scientist for so many discussions of details in the interpretation of spectra which arose during the experimental work for this paper.

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