

Primer-Like Inhibitors for DNA Repair Enzymes of the AML-HL60 and WERI-1A/Y79 Malignant Cells

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Abstract

A conventionally synthesized thio- and cyano-modified single-stranded poly(dNTP) sequences of different molecular sizes ($20n - 200n$) and the same lengths routine poly(dNTP) and poly(NTP) species were obtained through the good services provided by the Russian Federal Bioorganic Products Group and by the ThermoFischer, Inc., and then tested for their impact on catalytic activities of β -like DNA polymerases from chromatin of HL-60, WERI-1A and Y-79 cells as well as for the affinity patterns in DNAPol β -poly(dNTP)/(NTP) pairs, respectively. An essential link between the lengths of ultrashort ($50n - 100n$) single-stranded poly(dNTP) sequences of different structures and their inhibitory effects towards the cancer-specific DNA polymerases β has been found. A possible significance of this phenomenon for both DNA repair suppression in tumors and a consequent anti-cancer activity of the DNA repair related short poly(dNTP) fragments has been for the first time emphasized with a respect to their pharmacophore revealing potential. Thus, this work presents an experimental attempt to upgrade a contemporary attitude towards the DNA derived products applied for anti-cancer agenda, particularly, for acute myeloid leukemia and retinoblastoma cell DNA repair machinery breakdown. In this study, tumor specific DNA polymerases β were found of being the targets for attack promoted with the primer-like single-stranded DNA fragments followed by consequent cytostatic phenomena. A novel concept of the DNA related anti-cancer medicines is under discussion.

Keywords

Magnetic Isotope Effects (MIE), DNA Repair, DNA Polymerases, DNA-Enzyme Binding

“compartment” for this reaction (10 - 15 nm or less), all physical parameters describing a molecular machinery in normal and cancer cells at proliferation rates observed are identical or pretty close to each other [5]-[10].

Obviously, the DNA synthesis reaction [1] [2] [3] is unlikely a source for apoptosis of tumor cells as relates to dependence of their viability on MIE [1]. Nonetheless, this MIE-viability dependence might have a link to DNA repair activity expressed by beta-like DNA polymerases in neoplasma [2] [3] [11].

According to our assumption, these enzymes are to produce the ultrashort PDRN sequences ($\approx 40n$) playing a role of DNAPol β inhibitors. Acting as the DNA repair limiting/damaging factors, these endogenous inhibitors may promote an essential anti-cancer effect. In case of $^{25}\text{Mg}/^{43}\text{Ca}/^{67}\text{Zn}$ -induced impacts on enzymatic activity *in situ*, these inhibitors might be originated as a direct response to MIE.

Moreover, due to a random mode of the cancer-specific repair-required local DNA damages, the DNA repair itself means a wide variability in primary structures of the repair-related PDRN species. This also means that the PDRN inhibitory function should be determined predominantly, if not exclusively, by the lengths of these sequences. These lengths alone might determine not only the molecular dynamics in DNAPol β -PDRN docking pairs but, simultaneously, they might make an impact on specific Coulomb and dispersion couplings and, hence, on the mean amounts of hydrogen bonds per one nucleotide of the DNA primer inside the enzyme catalytic site.

So if this assumption is true, the DNAPol β activity must be “suppressible” by $\approx 40n$ -long polynucleotides regardless on their primary structures. This would make the MIE-promoting treatment a legitimate way to gain the *in situ* anti-cancer molecular events.

The aim of a present study is to test this hypothesis. For this purpose, several types of synthetic single-stranded polynucleotides (both DNA- and RNA-like ones) with molecular sizes ranged in $20n - 200n$ interval were investigated to reveal 1) their effects on catalytic activities of several beta-like DNA polymerases from HL-60, Y-79 and WERI-1A cancer cells, and 2) their enzyme-binding parameters. A selection of these peculiar cell models was determined solely by a sharp difference between the tumor proliferation rates, “aggressive” high (acute myeloid leukemia) and relatively low (retinoblastoma) [8] [9] [10] and, hence, by the difference in contributions made by the DNA repair key enzymes into the cancer cell survival capabilities.

2. Materials and Methods

2.1. Reagents and Disposal Materials

Aphidicolin (Fluka, Switzerland); ddTTP (Boehringer-Mannheim, Germany); Proteinase K (Serva, Germany); HSA (Sigma, USA); yeast tRNA (Serva, Germany); calf thymus DNA primer (Merk, Germany); dATP, dTTP, dCTP, dGTP (Serva, Germany); dioxane- and toluene-based media for liquid beta-scintillation

count MS460 and MS710 (Merk, Germany); CS20 25 mm fiberglass filters (Millipore, France); AccuPrep DNA extraction K2600 kit (Bioneer, Rep. Korea); [Methyl-1,2-³H]dTTP, 120 - 160 Ci/mmol (Amersham, UK).

2.2. Ligands

Poly(dT)₂₅, Poly(dT)₅₀, Poly(dT)₁₀₀, Poly(dT)₂₀₀ (ThermoFisher, USA); Poly(A)₄₉, Poly(2-thio-dC)₄₉, Poly(dT)₄₀ (IBC RAS, Russia); Poly(dT)₄₀, Poly(dT)₈₀ (ICBFM RAS, Russia); Poly([2-¹⁴C]CN-dT)₄₀, 20 - 38 Ci/mmol; Poly([2-¹⁴C]CN-dT)₈₀, 20 - 32 Ci/mmol; Poly([5-¹⁴C]CN-dT)₄₀, 18 - 24 Ci/mmol; Poly([5-¹⁴C]CN-dT)₈₀, 16 - 22 Ci/mmol (Perkin Elmer, USA).

2.3. Enzymes

PAGE-homogenous beta-like monomeric, 66.5 kDa and 23.5 kDa, DNA polymerases (EC 2.7.7.7) purified from chromatine of HL-60 acute myeloid leukemia cells [11] and WERI-1A, Y-79 retinoblastoma cells [2] were employed. Thus, to purify these chromatine-associated DNA repair programming enzymes, the following [2] [11]—promoted technique has been used. First, the chromatin fraction of the cell lysate was obtained. For this purpose, the protein containing crude nuclei phenol-chloroform extracts [2] [11] were mixed with 10 volumes of an ice-cold acetone and kept at +4°C overnight. An acetone-insoluble material was precipitated at 20,000 rpm, 20 min, +4°C. The pellets were extensively re-washed with acetone using the same procedure and then dissolved in 5 - 6 volumes (w/v) of 25 mM potassium-phosphate (pH 6.30)/0.5% NaCl/1.5 mM EDTA/0.01% glutathione/0.05% heparin/1.0% 2-mercaptoethanol/80 - 100 U/mL nuclease S followed by 40 min incubation at +37°C. All post-incubation mixtures were treated by sonication at 80 KHz, 30 min, +60°C, under a non-stop extensive shaking. Then these mixtures were submitted to a scalar fractionation path reaching the 30% - 70% ammonium sulfate saturation, consequently. The precipitates obtained were collected at 10,000 rpm, 20 min, and dissolved in 15 mM potassium phosphate buffer (pH 6.0)/0.2% NaCl (10 vols, w/v). The solutions were subjected to dialysis against 20 mM potassium phosphate buffer (pH 6.0) and lyophilized.

The lyophilized powders were first dissolved in 15 mM potassium phosphate (pH 6.30)/5.0 mM MgCl₂/1.5 mM EDTA/0.0001% sodium azide and passed through the fiberglass filters with 0.3 - 0.4 μ pore diameter (Millipore 5R, Millipore, France). The transparent solutions were subjected to ultrafiltration on membranes with the molecular size exclusion limit of 5.0 kDa at 800 p.s.i. (Diaflo Y5.0 25 mm membranes, Amicon BV, The Netherlands). The membrane-retained material was then extracted with 10 mM Tris-HCl (pH 8.0)/1.0% 2-mercaptoethanol (v/v), 5.0 mL per a razor-disintegrated membrane, +30°C, 12 hrs, with a following concentration in a rotor evaporizer.

The 1.5 - 2.5 mL samples were then applied onto a 1.5 × 50 cm (V = 98 mL) column packed with the TOYOPEARL HW 55F gel and equilibrated by the elu-

ent buffer consisting of 15 mM potassium phosphate (pH 6.30)/5.0 mM MgCl₂/0.0001% sodium azide. Elution rate: 0.8 mL/min (room temperature). In each one of the consequently eluted 1.5 mL fractions, the DNA polymerase activity has been measured according to [2] [11].

2.4. Enzyme Activity Estimation

Beta-like catalytic activity values were expressed in amounts of [3H]dTTP incorporated into nascent DNA chains in 1 min of incubation at optimal conditions corrected per 1.0 mg of pure enzyme protein ([3H]DNAcpm/mg protein) as described in [2] [11]. The enzyme catalytic activity was measured in 0.15 mL incubation mixtures consisting of 50 mM Tris-HCl (pH 8.0)/8.0mM dithiothreitol/15 mM MgCl₂/15% glycerol (v/v)/27.2 µg act DNA, calf thymus/50 µg each of dATP, dCTP, dTTP, dGTP/0.25 µmole [Methyl-1,2-³H]dTTP (90 - 120 Ci/mmol, NET520A, NEN)/150 mM NaCl. The tritium-labeled nucleotide was purchased from New England Nuclear, USA. These compound concentration values were first pre-optimized within both pH 6.0 - 9.0 and 5.0 mM - 50.0 mM MgCl₂ ranges. These mixture samples were first pre-incubated at +37°C for 60 min. Then 5.0 - 7.5 µg of pure enzyme was added to each one of these running samples and they were incubated at +37°C for 60 min longer. The ice cold incubation samples (0°C, 60°C after pre-incubation) as well as the trypsin treated samples (20 µg/mL trypsin, Merck GmbH, Germany, +37°C, 60°C) were taken for controls.

The post-incubation mixtures were subjected to a quantitative extraction of the DNA ultramicro-amounts using an AccuPrep Genomic DNA Extraction Kit (Bioneer Corp., Korea).

2.5. DNA and Protein Measurements

The DNA ultramicro amounts measurements were performed in diluted water solutions according to [12] [13]. The protein ultramicro amounts were estimated by method [14] modified in [12].

2.6. Radioactivity Measurements

For [³H] and [¹⁴C] radioactivity quantitative detection, the cpm values were determined using the DNA-retaining fiberglass filters (DNApolβ post-incubation ethanol-precipitation pellets) [11] placed into dioxan or tolyene media [2] [3] [11] with a following processing in Wallac 2200LX liquid scintillation counter (Wallac, Finland).

2.7. Ligand-Enzyme Binding Measurements

To quantify a ligand-enzyme binding patterns, a conventional criteria such as K_{dis} (K_d) and A_c were taken into account.

A Baileyan model was applied for an algorithm leading to a ligand-enzyme (ligand-receptor) dissociation constant estimate [7] [15].

In this approach, a ligand-receptor interaction $R + L \xrightleftharpoons[\beta]{\alpha} RL$, where R is the receptor, L is the ligand, RL is the ligand-receptor complex, α is the probability of formation of a complex molecule, and β is the probability of its dissociation. If the random number of ligand-receptor complex molecules is x , and the initial number of receptors is R_0 , the number of free receptors makes $R_0 - x$. Assume that the process unfolds under the condition of large ligand surplus, so that the number of ligand molecules stays equal to its initial value L_0 . The formation of ligand-receptor complexes is described by the function $f_1 = \alpha L_0 (R_0 - x)$, and their decomposition—by $f_{-1} = \beta x$. Bailey's equation system is:

$$\begin{aligned} \frac{\partial M(\theta, t)}{\partial t} &= L_0 R_0 \alpha (e^\theta - 1) M(\theta, t) - L_0 \alpha (e^\theta - 1) \frac{\partial M(\theta, t)}{\partial \theta} \\ &\quad + \beta (e^{-\theta} - 1) \frac{\partial M(\theta, t)}{\partial \theta} \\ \frac{dk_1(t)}{dt} &= L_0 R_0 \alpha - L_0 \alpha k_1(t) - \beta k_1(t), \\ \frac{dk_2(t)}{dt} &= L_0 R_0 \alpha - L_0 \alpha k_1(t) - 2L_0 \alpha k_2(t) - \beta k_1(t) + 2\beta k_2(t), \\ k_1(t) &= m(t) = \frac{\beta l r}{\beta l + \alpha} (1 - \exp[-(\beta l + \alpha)t]), \\ k_2(t) &= \sigma^2(t) = \frac{\alpha \beta l r}{(\beta l + \alpha)^2} (1 - \exp[-(\beta l + \alpha)t]) \\ &\quad + \frac{\beta^2 l^2 r}{(\beta l + \alpha)^2} \exp[-(\beta l + \alpha)t] (1 - \exp[-(\beta l + \alpha)t]) \end{aligned}$$

Following this algorithm, an experimental data on the ligand-enzyme complexes stability in water solutions obtained by techniques described in [7] [15] and modified in [16], and affected by temperature/ultrasound/ionic strength were processed using a LQ170 SigmaLab software in HP9000 analytical system (Hewlett Packard, USA).

For a non-specific binding control, HSA and denaturated-renaturated misfolded yeast tRNA were employed as the pseudoligands [17] [18].

In all enzyme-ligand coupling tests, the enzyme required optimal catalytic parameters [2] [11] were held for 40 min, +37°C, while the following ligand concentrations kept at 0.1 μ M, 0.5 μ M, 10.0 μ M, 20.0 μ M, 50.0 μ M, as recommended in [15] [17].

The UV-spectrophotometry (Lambda 1050 Scanning Spectrophotometer, Perkin Elmer, USA) was employed using the Spectra Manager II cross-platform software (JASCO, USA) for automated data treatment with an aim to gain the A_c index indicating an extent of polynucleotide release from ligand-enzyme in water solution owing to a certain consent of A_{210} , A_{254} , A_{280} values,

$$A_c = \left[\frac{A_{254}}{A_{280} - A_{254}} \right] / A_{210} \quad [16].$$

2.8. Statistics

A Dunnett's non-parametric ($n \leq 6$) technique was used to elucidate reproducibility of the data along with a significance of differences in control-experiment comparisons [19].

3. Results

A variable set of polynucleotide ligands (see Methods) was tested for both inhibitory effects and the ligand-enzyme binding properties using beta-like DNA polymerase species from acute myeloid leukemia (HL-60) and retinoblastoma (WERI-1A, Y-79) cells.

As seen from the data presented in **Figure 2** and **Figure 3**, no matter what ligand concentration tested and whatever enzyme sample coupled, a molecular size of synthetic polynucleotide is the only, crucial, impact-making factor. It should be emphasized that a maximal inhibition effects were observed in cases of 50n - 100n-long polynucleotides (**Figure 2(C)** and **Figure 2(D)**), **Figures 3(A)-(E)**), while a post-denaturation misfolded tRNA test show no inhibition

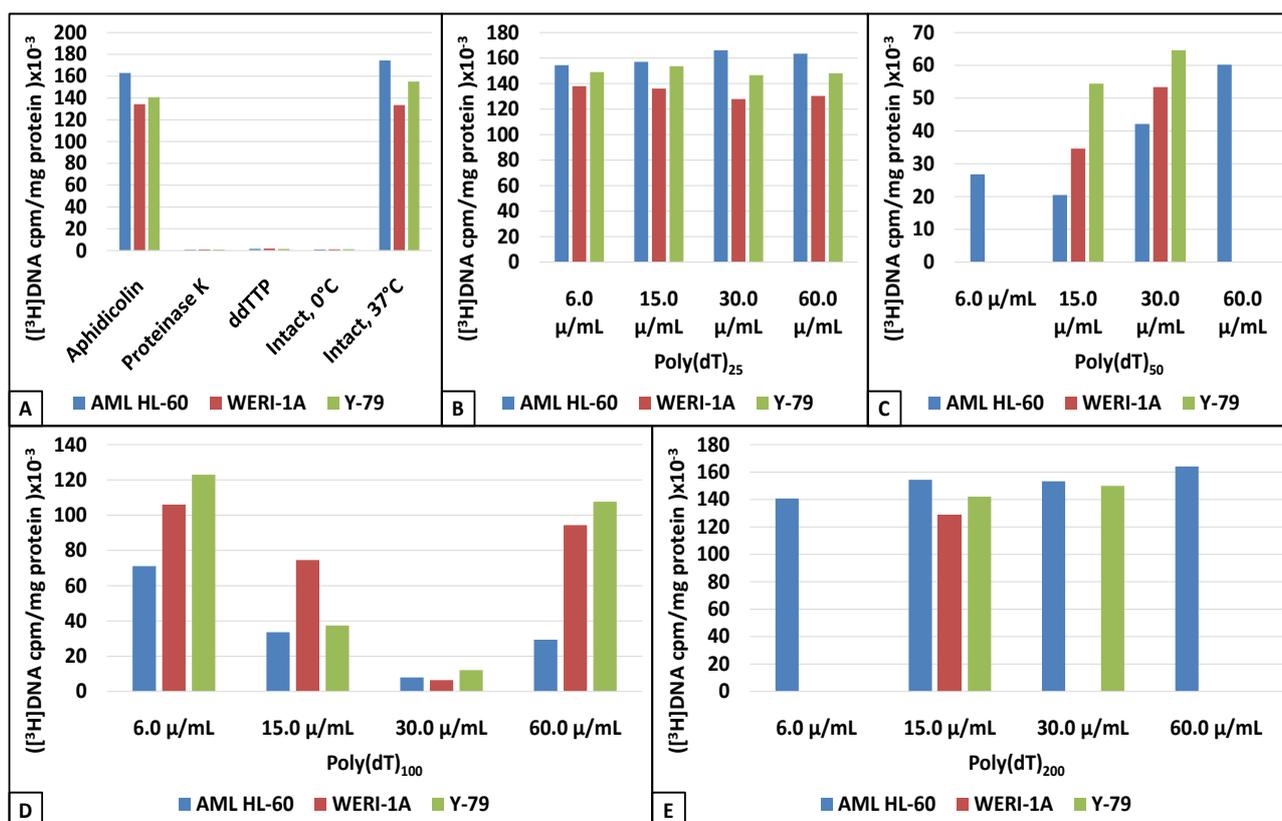


Figure 2. (A) Catalytic activities of beta-like DNA polymerases from HL-60, WERI-1A and Y-79 cancer cells under optimal and modified incubation conditions. (B) Catalytic activities of beta-like DNA polymerases from HL-60, WERI-1A and Y-79 cancer cells in the presence of Poly(dT)₂₅. (C) Catalytic activities of beta-like DNA polymerases from HL-60, WERI-1A and Y-79 cancer cells in the presence of Poly(dT)₅₀. (D) Catalytic activities of beta-like DNA polymerases from HL-60, WERI-1A and Y-79 cancer cells in the presence of Poly(dT)₁₀₀. (E) Catalytic activities of beta-like DNA polymerases from HL-60, WERI-1A and Y-79 cancer cells in the presence of Poly(dT)₂₀₀.

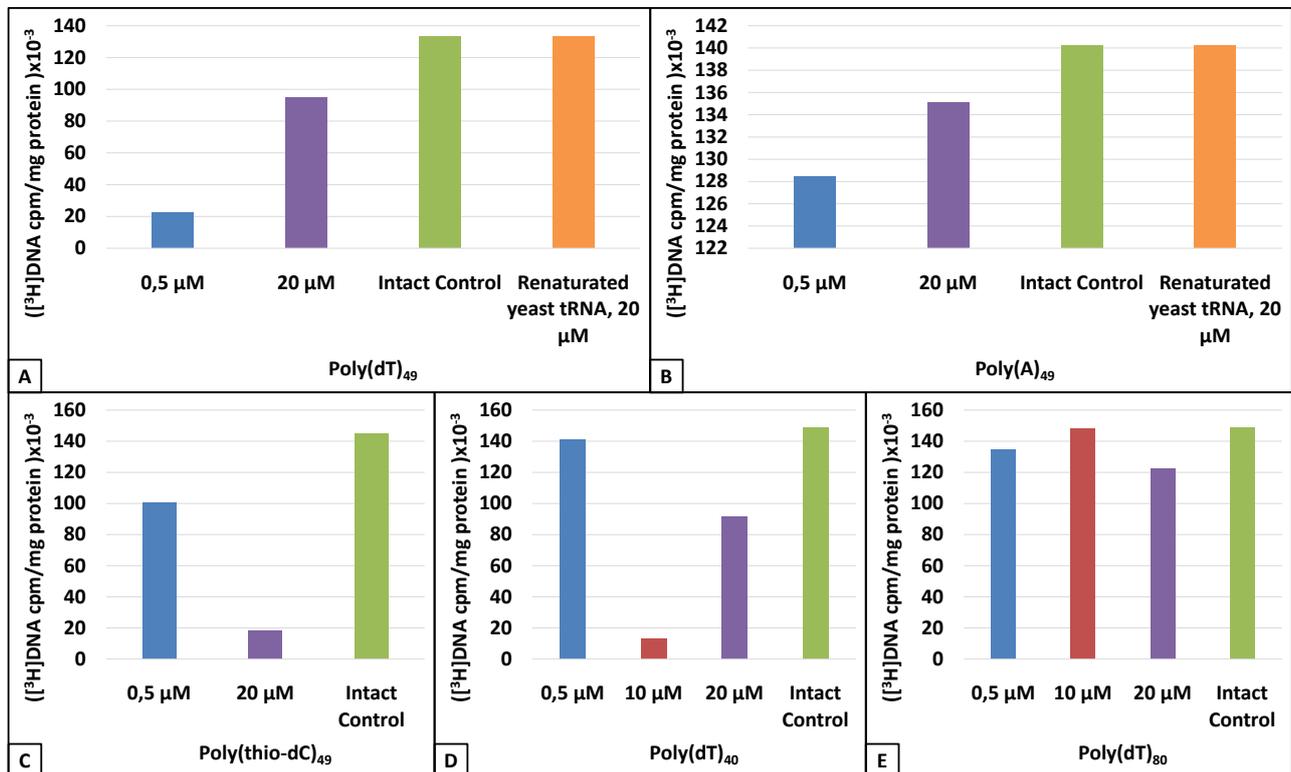


Figure 3. (A) Catalytic activity of AML/HL-60 beta-like DNA polymerase in the presence of Poly(dT)₄₉ and renaturated yeast tRNA. (B) Catalytic activity of AML/HL-60 beta-like DNA polymerase in the presence of Poly(A)₄₉ and renaturated yeast tRNA. (C) Catalytic activity of AML/HL-60 beta-like DNA polymerase in the presence of Poly(thio-dC)₄₉ and renaturated yeast tRNA. (D) Catalytic activity of AML/HL-60 beta-like DNA polymerase in the presence of Poly(dT)₄₀. (E) Catalytic activity of AML/HL-60 beta-like DNA polymerase in the presence of Poly(dT)₈₀.

effect at all (**Figure 3(A)** and **Figure 3(B)**). As per Aphidicolin and ddTTP controls, they just prove a true beta-specific nature of enzymes tested [2] [3] [11] (**Figure 2(A)**).

Unlike polydeoxyribonucleotides (PDRN), polyribonucleotides were found to be inert towards the DNAPol β processivity regardless on lengths of these ligand molecules (**Figure 3(A)** and **Figure 3(B)**).

The affinity isotherms clearly show an essential difference between the ligand-enzyme complex stability patterns, K_d and A_c (see Methods), estimated for numerous compositions of these couples (**Figure 4** and **Figure 5**). Thus, dissociation constant K_d for Poly(dT)₅₀-DNAPol β was found by 2.5-fold smaller as compared to Poly(dT)₂₀₀-DNAPol β pair (**Figure 5**).

In all cases studied, except for the RNA-like Poly(A)₄₉-containing pairs (**Figure 5**), K_d and A_c values are minimal when the ligand length is close to 50n (**Figure 6**). For all RNA-like ligands, the high values of K_d and A_c were found practically equal to the ones estimated for Poly(dT)₂₀₀ (**Figure 5**).

The data presented in **Figure 2** and **Figure 3** leaves no doubt about a maximal inhibitory efficiency provided by ultrashort, 40n - 50n in length, cyano-polydeoxyribothymidyl derivatives. These agents are to show as high as nearly 6.5-fold extent of enzyme suppression. That does not mean, however, that

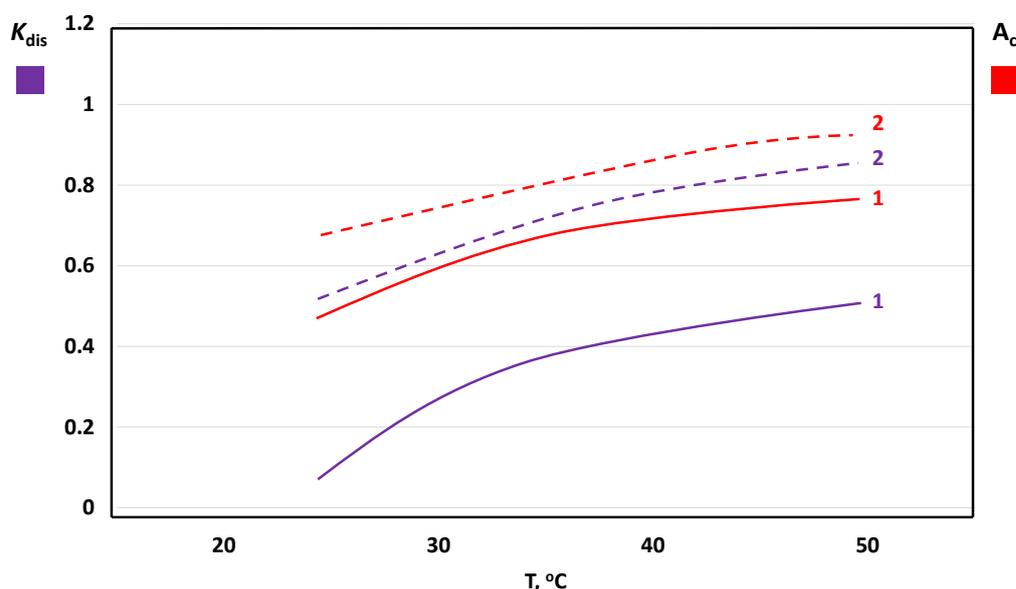


Figure 4. Affinity isotherms for HL-60-DNApol β /Poly(dT)₅₀ (1) and HL-60-DNApol β /Poly(dT)₂₀₀ (2) ligand-acceptor complexes. For technical details, see Methods.

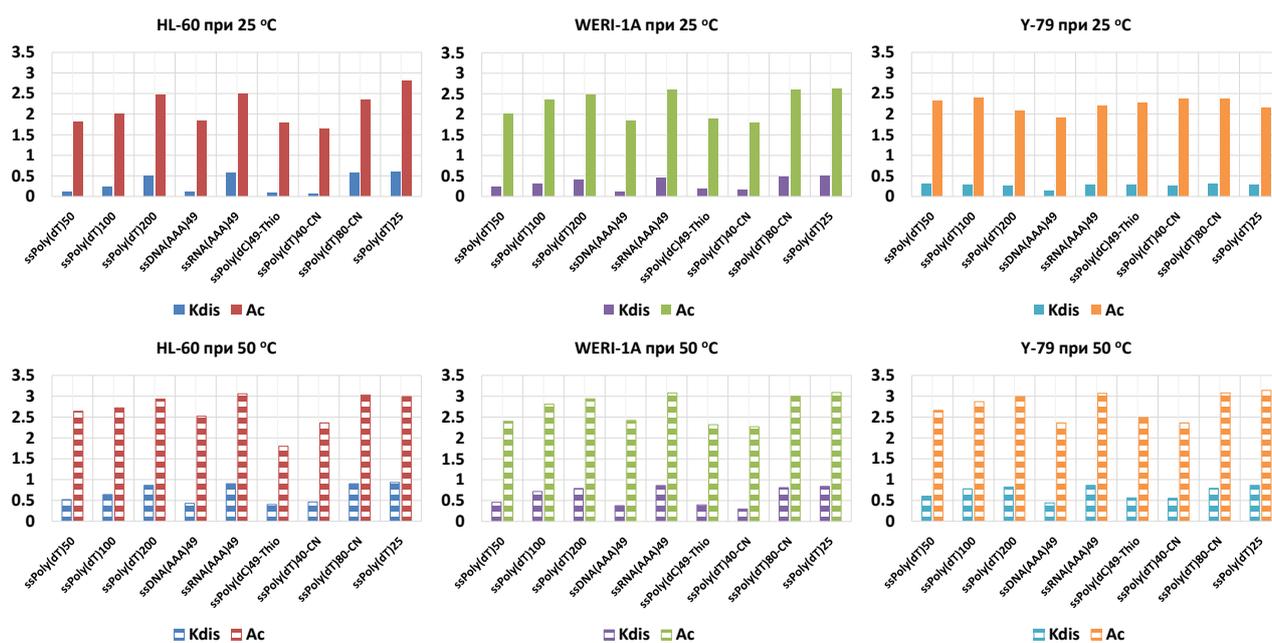


Figure 5. Polynucleotide-enzyme binding patterns (K_{dis} , A_c) estimated for beta-like DNA polymerase species from three cancer cell lines (HL-60, WERI-1A, Y-79). For technical details, see Methods.

this particular type of “pseudoprimers” is the only one capable to play a role of a powerful DNApol β inhibitor. A truly crucial parameter to reveal a potential pharmacophore signs have nothing to do with a structure of DNA fragments being entirely related to its molecular size (Figure 2 and Figure 3).

So the results observed looks worthy of not indicating to any particular and perfect pharmacophore found but, instead, of a detailed analysis as a new platform to upgrade a whole approach towards the DNA derivatives in a current

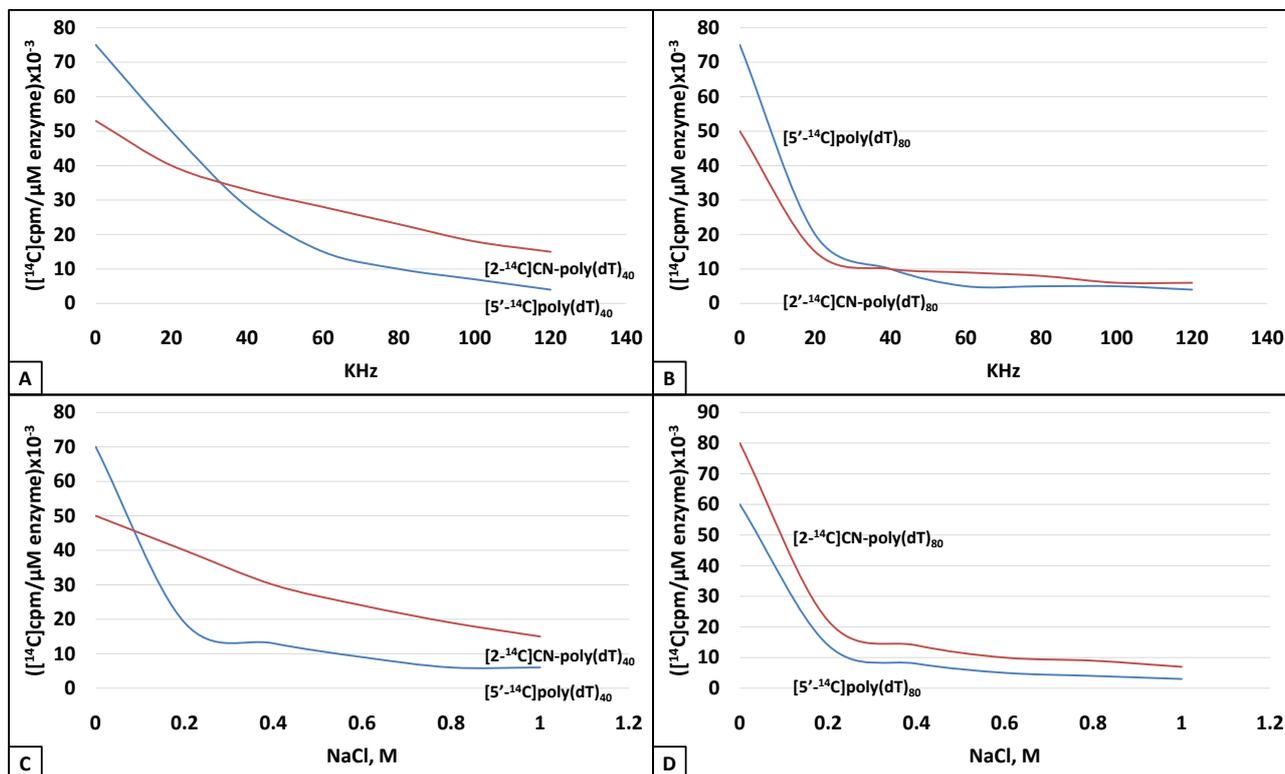


Figure 6. (A) A sonication affected stability of the couples of HL-60 beta-like DNA polymerase and ^{14}C -labeled Poly(CN-dT)₄₀ evaluated by detection of free ligand release. *For technical details, see Methods.* (B) A sonication affected stability of the couples of HL-60 beta-like DNA polymerase and ^{14}C -labeled Poly(CN-dT)₈₀ evaluated by detection of free ligand release. *For technical details, see Methods.* (C) A salt-caused decay of the unstable complexes between HL-60 beta-like DNA polymerase and ^{14}C -labeled Poly(CN-dT)₄₀ evaluated by detection of free ligand release. *For technical details, see Methods.* (D) A salt-caused decay of the unstable complexes between HL-60 beta-like DNA polymerase and ^{14}C -labeled Poly(CN-dT)₈₀ evaluated by detection of free ligand release. *For technical details, see Methods.*

anti-cancer agenda. This a Discussion of our work is all about.

4. Discussion

A wide diversity of investigated synthetic homopolynucleotides and their monotonic thio- and cyano-derivatives is itself a prove for universal, indiscriminate, mode of DNAPol β inhibition effect we described. This is in a favor with our assumption stated that the key role in this phenomenon belongs exclusively to molecular sizes of small DNA fragments regardless on their primary structure peculiarities.

The ligands diversity mentioned makes no way to explain a formation of the ligand-enzyme complexes just by a routine docking (“complimentary”) paradigm. This DNA-protein binding act may involve some random appearing hydrogen bonds, Coulomb hyperfine coupling along with dispersion interaction and, last not least, the hydrophobic connections, might also be a case in this scenario. A key point of this model derives from the homopolynucleotides related data and reveals that the mean energy of these “weak” interaction depends on the length of a ligand but not of its structure.

A similar event occurs in the DNA repair process as long as a randomly appeared local DNA damage comes first. In this case, a clearly indiscriminate mode of the ligand-enzyme coupling is a consequence of unpredictable variability in primary structures of DNA fragments suitable for the repair purposes.

Therefore, a polynucleotide length alone is an impact-making factor towards the DNAPol β catalytic function. This factor might determine a formation of the above listed “weak” non-covalent interactions and, hence, to control the mean amount of hydrogen bonds per one nucleotide of DNA-primer (ligand) inside the DNA polymerase catalytic site. That is indeed critical for the enzyme suppression *in situ*.

All the affinity isotherms presented and a variable ligand-enzyme binding data (Figures 4-6) reveals a maximal strength of this peculiar intermolecular interaction within the short interval of ligand sizes, $50n - 100n$. This is to confirm that the enzyme inhibition we observed (Figures 2(B)-(E), Figure 3) is indeed a consequence of the stable ligand-enzyme pair formation which has nothing to do with either the solution properties changes or the enzyme surrounding disperse phase modulations.

A comparison between the ligand amounts bound with (a) DNAPol β and (b) HSA show that the ligand-enzyme complex includes two molecules of ligand per one molecule of enzyme, while the ligand-HSA (control) pair contains just one ligand per HSA molecule (Figure 4, Figure 7, Figure 8). This is in a favor to a mere fact of existence of two separate Mg $^{2+}$ -coordinating catalytic sites in β - and β -like DNA polymerases [8] [10] [20] [21] [22].

Let's evaluate the activation energy value for a ligand-enzyme complex dissociation following the data listed in Figure 4 and Figure 8. Obviously, a

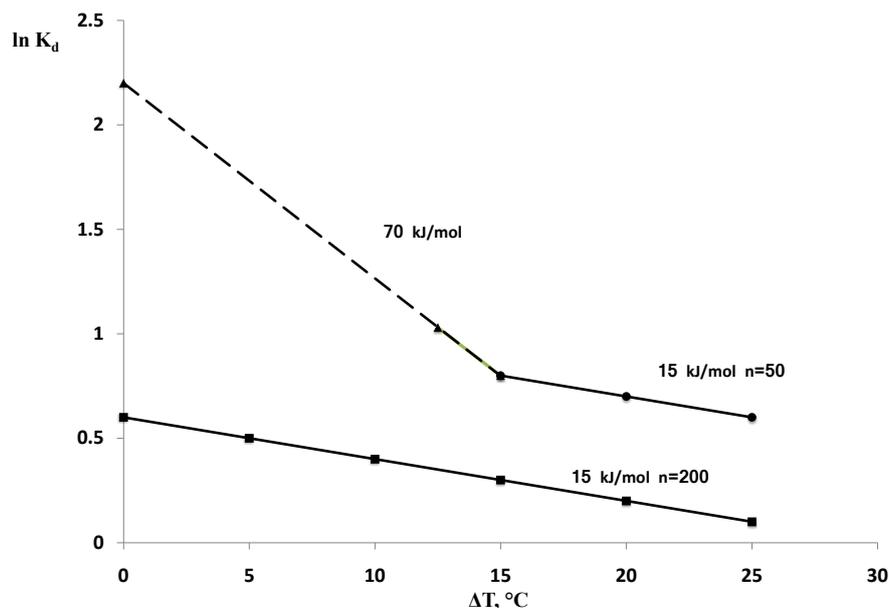


Figure 7. An activation energy of the ligand-enzyme binding as affected by temperature in DNAPol β -HL60/Poly(dT) $_{50}$ pair formation. $\ln K_d \cong \ln K_0 - E/RT_0 + E\Delta T/RT_0^2$. See Discussion.

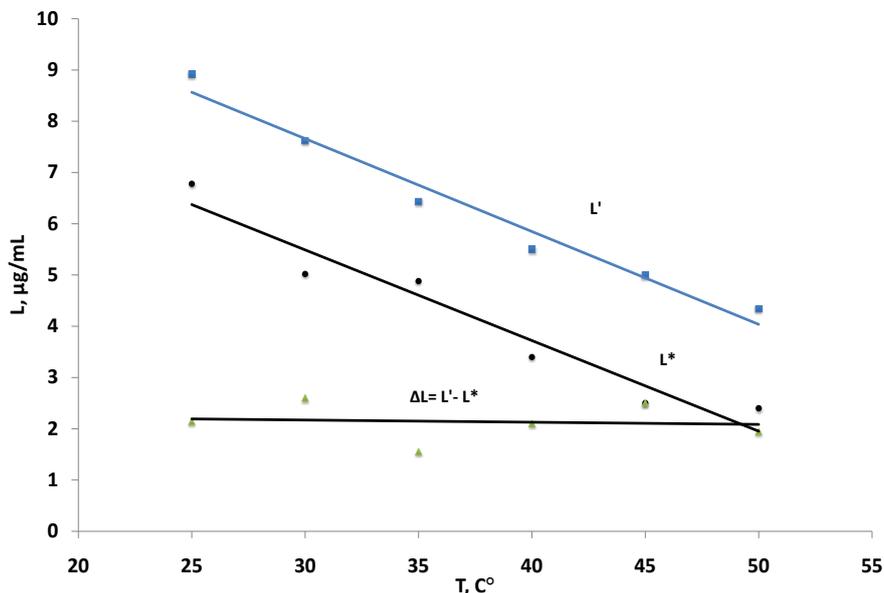


Figure 8. Temperature-dependent ligand binding in DNAPolβ-HL60/Poly(dT)₅₀ pair. L'—DNAPolβ, receptor; L*—HAS, receptor (control). For technical details, see Methods.

dependence of K_d on temperature has a clear Arrhenius mode:

$$K_d = K_0 \exp(-E/RT) \quad (1)$$

where R —a universal gas constant and T —enzyme functioning temperature.

Taking into account a low level of the temperature interval tested as compared to an initial $T_0 = 298\text{K}$, we'll use further a linear decay as T in (1). To estimate E , a linear regularity must be treated as:

$$\ln K_d \cong \ln K_0 - E/(RT_0) + E\Delta T/RT_0^2 \quad (2)$$

where ΔT is a temperature change in experiment (Figure 7 and Figure 8).

The Equation (2) revealed activation energy value E was found 15 kJ for Poly(dT)₂₀₀ ligand within a whole range of temperatures tested, while the very same dependence for Poly(dT)₅₀ include two linear branches crossing each other nearby the 40°C point where, most likely, a slight protein denaturation starts (Figure 7). Once the temperature is close to an enzyme function required optimal level (25°C - 35°C), the Equation (2) revealed $E_{50} = 70$ kJ/mole whereas this value estimated at 40°C is as low as 15 kJ/mole (Figure 7). The attention catching point here is that the E value measured at the enzyme denaturation launch-point (40°C) is exactly the same as it has been found in case of the “inert”, non-inhibiting, polydeoxyribonucleotide ligands. This supports a statement on the enzyme inactivating protein structure dynamics initiated starting with a 40°C heating point. A lack of two-compartment, “angle-breaking”, $K_d = f(T)$ dependence for long ligands and their E values low level are in a good accordance with a high probability of 3D-compactization of such sequences at our test conditions [7] [16] [17] [23].

It's easy to find out that the inhibition-promoting Poly(dNTP) species provide

a pretty low value of activation energy for dissociation with DNAPol β -HL60, $\varepsilon = 1.40$ kJ/mole. That means, the complimentary hydrogen bonds do not contribute to formation of these particular Poly(dNTP)-enzyme complexes which is a prove for the absence of the affinity-specific, true docking links in these pairs. Most probably, this remarkable 1.40 kJ/mole value is determined by the U_d -dispersion related weak Van der Waals interactions along with a U_q Coulomb hyperfine coupling. These kinds of the ligand-enzyme complex stabilizing links are rather superficial ones due to both a $U_d \sim 1/r^6$ potential and the efficient screen-like separation of Coulomb forces in water occurred within a distance smaller than 0.4 nm [15] [17] [23] [24]. This certainly takes into account the superficial hydrophobic bounds as well which are nothing but the result of a cooperative (cumulative) act of all the above specified forces [23] [24].

So the key role in formation of Poly(dNTP)/DNAPol β complexes belongs to Van der Waals bounds which energy corresponds directly to either 1) the efficient surface of ligand-enzyme pair or 2) a length of an enzyme-bound ligand.

Furthermore, since the mean value of the U_d dispersion patterns is hardly dependent on the element composition, and as long as the amounts of charged groups in U_q -determining Poly(dNTP) ligands tested are about the same, a very similar parameters of their inhibitory activity should be expected then. This explains well a lack of dependence of inhibitory capabilities of these ligands on their nucleotide composition (**Figures 2(B)-(E)**, **Figure 3**).

Once $\varepsilon \sim RT$, we might suppose that the short ligands (50n - 100n, or shorter) are to get bound with the enzyme molecule throughout a whole linear chain, *i.e.* through nearly every nucleotide of Poly(dNTP) sequence.

Using the Arrhenius equation, we may evaluate a ratio between the ligand-enzyme binding time values τ for Poly(dNTP)₂₀ and Poly(dNTP)₅₀, respectively:

$$\tau_{20}/\tau_{50} = \exp(-\Delta n\varepsilon/RT) \cong 10^{-7} \quad (3)$$

where $\Delta = 30$ (difference in lengths between 50n and 20n). It shows that a short time of binding τ_{20} does not allow n_{20} -ligand to promote a sharp suppression of the enzyme activity. A shorter time for the ligand “neighboring” around the enzyme catalytic site, the lesser inhibitory effect to be expressed.

On other hand, the longest ligands tested were found to be a rather poor inhibitors as well (**Figure 2(E)**, **Figure 4**) due to their compactization-caused small square of the enzyme-ligand efficient interaction [23] [25] as also seen from Equation (3).

As per a zero inhibition affect shown by RNA-like ligands tested (**Figure 3(A)** and **Figure 3(B)**), their remarkable refolding capabilities [18] [25] and [-O-----Mg²⁺] coordination input [26] [27] are presumably beyond this phenomenon.

A clear pharmacological potential of oligo- and polydeoxyribonucleotides is a subject for numerous experimental and clinical studies performed since early 1990s [18] [27] [28] [29] [30]. Their applied medicinal significance, however, were treated predominantly by focusing on the aptomer properties, *i.e.* on capa-

bilities to find the specific target molecules such as signaling proteins, membrane receptors, chromatin structure elements, etc [17] [26] [28] [30] [31]. Unlike our present work, therefore, these studies are all about the true docking participants. The inhibitory power of Poly(dNTP) species we found has nothing to do with their structures and composition being entirely dependent on molecular size (Figures 2(B)-(E), Figure 3(A), Figure 3(C), Figure 3(D), Figure 3(E)). This allows to “turn the page” in a long record of polynucleotide medicinal applications by just paying attention to the Poly(dNTP) motion on how to shut down a DNA repair in malignant cells. It makes sense for DNAPol β , key DNA repair enzymes, are found hyperexpressed in response to a high frequency of DNA damages followed by an increase of the cancer related cell proliferation rate [2] [3] [10] [11] [20]. With a respect to this statement, it has been already emphasized that the members of this enzyme family are the “legitimate targets” for cytostatic inhibitors like dNTP antimetabolites [8] [9] [10] [28] [32] and magnetic metal isotopes [1] [2] [3] [24].

There is a good enough understanding on molecular mechanisms beyond the inhibition of the cancer-hyperexpressed DNAPol β species provided by magnetic isotopes of bivalent metals— ^{25}Mg , ^{43}Ca and ^{67}Zn [1] [2] [3] [9] [24]. In particular, these mechanisms are dealing with the origin of “too short”, DNA repair insufficient, DNA fragments [1] [2] [3]. This effect leads to a massive release of the DNA repair related $40n - 100n$ DNA sequences with the unpredictable, unique structures [1] [2], *i.e.* this provokes a magnetic isotope induced formation of the DNAPol β endogenous inhibitors. It looks like a good explanation for cytostatic activity shown by magnetic metal isotopes in several cancer cells [1] [24].

Everything we know about pharmacokinetics of various DNA-based drugs and pharmacophores indicates to the fact that their bioavailability is far of being perfect mostly because of inevitable nucleases attack [18] [27] [28] [29] [30]. So this is the hardest task to manage a targeted delivery of Poly(dNTP) sequences to the DNAPol β -operating *in situ* compartments, for which some certain nanocarriers required [7] [18] [30] [33]. As for the magnetic metal ions, they can be delivered to the DNAPol β *in situ* targets with [1] [24] or even without [2] [3] nanocationite administration which would make the magnetic isotope effect a promising tool to gain the DNA repair machinery breakdown in neoplasma.

Knowing that the abundance of ^{25}Mg is relatively high ($\approx 10\%$) and considering a remarkable role of magnesium in enzymatic phosphorylation processes [1] [24] [34], it would be logical to suppose the existence of so called “hidden” effects of Magnesium-25 on numerous metabolic pathways. In this case, a high content of endogenous Fe^{2+} in cells and tissues of a living organism becomes a natural limiting factor to prohibit any expression of magnetic isotope effects *in vivo*. This is in accordance with the data on correlations between the $^{25}\text{Mg}^{2+}$ -affected ATP synthesis levels in mitochondria and the Fe^{2+} contents in these mitochondria isolated from different rat tissues [34] as well as with the results stated an increase of Fe/Mg ratios in several types of cancer (ovarian cancer, renal adeno-

carcinoma, fibroblast lung cancer, hepatocellular cancer, osteosarcoma, thyroid follicular adenocarcinoma) compared to corresponding normal, non-malignant, tissues [9] [20] [26] [32].

A variety of clinical case reports showing an appearance of short ($100n - 300n$) single-stranded DNA fragments in a blood plasma of oncology patients [26] [35] may now also be treated under a point of view of the probable DNA repair related origin of these tumor-released Poly(dNTP) sequences.

Last not least, the infamous quorum sensing phenomena [36] could be engaged with an effort to uncover a biological meaning of what we've found testing the capabilities of different Poly(dNTP) species to affect some tumor-specific DNA repair key enzymes.

5. Conclusions

Ultrashort ($40n - 100n$) polydeoxyribonucleotides are found to be the efficient non-specific inhibitors of DNA polymerases β from chromatin of several cancer cells: HL-60, WERI-1A, Y-79.

Manifesting a positive correlation between these inhibitory capabilities and the strength of affinity in enzyme-ligand pairs, such poly(dNTP) species promote a sharp suppression of enzymatic catalysis regardless on their primary structures. Only molecular size matters.

The data presented show a clear regulatory potential of some short ssDNA entities once they are about to interact with the key DNA repair enzymes.

This may provide a promising platform for further research aiming to shut down the DNA repair machinery in malignancies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

ssDNA, Single-Stranded DNA;
DNAPol β , DNA Polymerase Beta;
MIE, Magnetic Isotope Effects;
AML, Acute Myeloid Leukemia.