

Reviewer's Comment:

The manuscript was revised but it still has many misspellings, unclear sentences, and mistakes. In addition, various commentaries were not well addressed.

- Misspellings and unclear sentences:

Abstract

"72 cancer patients who administered chemotherapy with regiment which contains cyclophosphamide..." it should be replaced by "72 cancer patients receiving four or more cycles of cyclophosphamide"

"The results of the study showed that O⁶-methylguanine is not always found in patients suffering from cancer treated with cyclophosphamide". The sentence should be: The results of this study showed that O6-methylguanine is not always found in cancer patients treated with cyclophosphamide

"...the therapeutic dose given can be monitored and..." It should be: the dose administered should be monitored and...

- Commentaries 8 and 9 were not well addressed.
- Ref. 13 has mistakes. The year is 1999 and the authors are two, thus, et al should not be included.
- This reviewer still considers that the method should be tested.
 - ✓ Is O⁶-methylguanine stable in formic acid (90 % v/v) at 80°C for 1 hour?
 - ✓ Is there the possibility that a quantity of the analyte is lost by the effect of the acid (the authors used 90 % v/v formic acid) and temperature? According to the authors: "The DNA solution (200 µL) was mixed with HPLC grade water and formic acid (90%) in equal volume. The solution was mixed and heated at 80 °C for 60 minutes using a Thermomixer (Eppendorf) [19]. The resulting hydrolyte was injected into the UPLC-MS/MS."

E S Sadr developed in 2012 a method to quantify and detect N7- and O6-methylguanine adducts by liquid chromatography and mass spectrometry (The thesis is free and can be accessed at http://digitool.library.mcgill.ca/webclient/StreamGate?folder_id=0&dvs=1410369629678~690) and says: "Between 2 and 3 µg of DNA were used for each sample to detect N7-MeG and O6-MeG levels. Samples were heated at 85 °C in 10% (v/v) formic acid for one hour."

✓ How many replicates did the authors perform?

Overall, this referee considers that the manuscript should be improved.

Author's feedback:

All the misspellings and unclear sentences in the ABSTRACT are noted thus have been corrected.

8) Noted. I already provide the narration regarding the patient's characteristic. doses. drugs. and cycles in the manuscript because it is a long table that can be described in a succinct narration. I provide the complete table below as requested by the referee.

No	Diagnosis	Sex/ Age (yo)	Weight (kg)/Body Mass (mm ²)	Chemotherapy Protocol	Undergone Therapy Cycles	Last Cyclophosphamide Dose (mg)	Duration of Chemotherapy	Total isolated DNA (µg/mL)		Level of O ⁶ - methylguanine (ng/mL)	Note
1	Breast Cancer	F/53	67/1.747	FAC	5	1000	21 days	216.6	-	0.8010	Not detected
2	Breast Cancer	F/54	64/1.650	FAC	6	790	21 days	192.4	168.9	0.6787	Not detected
3	Breast Cancer	F/52	48/1.350	FAC	5	677	29 days	164.3	191.3	0.7279	Not detected
4	Breast Cancer	F/67	43/1.270	C-docetaxel	6	700	21 days	104.6	90.8	0.8644	Not detected
5	Breast Cancer	F/73	45/1.360	CMF	4	1000	21 days	246.3	232.6	1.2453	Detected
6	Breast Cancer	F/50	50/1.47	FAC	6	735	21 days	76.7	115.1	1.0386	Not detected
7	Breast Cancer	F/36	56/1.57	FAC	6	750	21 days	514.6	523.2	0	Not detected
8	Non-Hodgkin lymphoma	M/54	68/1.70	R-CHOP	6	1300	21 days	204.6	204.7	0	Not detected
9	Non-Hodgkin	M/56	55/1.588	R-COP +	6	1400	29 days	171.8	188.5	0	Not detected

	lymphoma			epirubicin							
10	Breast Cancer	F/49	58/1.48	FAC	5	750	29 days	192.5	190.0	0	Not detected
11	Breast Cancer	F/58	62/1.60	C-Docetaxel	6	1000	21 days	297.4	323.2	0	Not detected
12	Breast Cancer	F/60	55/1.57	CMF	5	750	21 days	170.9	148.8	0	Not detected
13	Breast Cancer	F/56	60/1.60	C-docetaxel + herceptin	6	804	21 days	200.6	203.0	0	Not detected
14	Breast Cancer	F/64	58/1.55	FEC	4	800	21 days	91.3	88.8	0	Not detected
15	Breast Cancer	F/48	73/1.73	FAC	6	845	21 days	267.4	265.1	0	Not detected
16	Breast Cancer	F/42	49/1.47	C-docetaxel	6	700	21 days	275.7	290.1	0	Not detected
17	Breast Cancer	F/40	39/1.66	C-docetaxel	6	900	21 days	368.4	339.4	0	Not detected
18	Acute Lymphoblastic Leukimia	M/3	12/0.653	C + methotrexate. vincristine. dexamethasone. mesna	4	500	21 days	202.0	202.3	0	Not detected
19	Non-Hodgkin lymphoma	M/46	50/1.78	R-CHOP	4	1200	21 days	64.4	67.9	0	Not detected
20	Non-Hodgkin lymphoma	M/50	81/1.83	R-CHOP	5	1600	21 days	149.2	149.8	0	Not detected
21	Burkitt Lymphoma	M/11	45/1.25	C+Vincristine. methotrexate. mesna	5	1200	21 days	288.3	270.1	0	Not detected
22	Breast Cancer	F/68	65/1.51	C-docetaxel	5	736	21 days	637.0	652.8	0	Not detected
23	Primitive Neuroectodermal Tumour	M/3	11/0.485	C+Vincristine. 5-fluorouracil	4	576	21 days	321.7	301.1	0	Not detected
24	Breast Cancer	F/48	62/1.69	C-paclitaxel	4	1002	21 days	165.3	164.2	0	Not detected
25	Breast Cancer	F/42	53/1.64	C-docetaxel	6	700	29 days	223.9	146.6	0	Not detected
26	Breast Cancer	F/45	64/1.596	FAC	4	798	21 days	122.1	119.9	0	Not detected
27	Breast Cancer	F/57	62/1.33	C-docetaxel	5	800	21 days	39.4	33.0	0	Not detected
28	Non-Hodgkin lymphoma	F/51	63/1.694	COP	4	1300	29 days	171.2	170.7	0	Not detected
29	Breast Cancer	F/40	63/1.55	AC	5	930	21 days	21.1	46.7	0	Not detected
30	Non-Hodgkin lymphoma	M/50	73/1.81	R-CHOP	5	1600	21 days	128.1	126.0	0	Not detected

31	Non-Hodgkin lymphoma	F/22	50/1.57	COP	4	1100	29 days	516.1	515.2	2.2679	Detected
32	Non-Hodgkin lymphoma	M/46	52/1.62	C+Vincristine. epirubicin	6	1200	21 days	465.2	434.4	0	Not detected
33	Acute Lymphoblastic Leukimia	F/7	20/0.77	C+Vincristine. adriamycin. mesna	5	525	21 days	104.6	103.7	0	Not detected
34	Breast Cancer	F/36	41/1.33	C+adriamycin. 5-fluorouracil	4	665	21 days	112.6	111.8	1.7729	Detected
35	Breast Cancer	F/51	81/1.51	C-Docetaxel	5	890	21 days	203.4	210.5	0	Not detected
36	Breast Cancer	F/42	36/1.212	FAC	4	607	21 days	119.7	120.4	0	Not detected
37	Breast Cancer	F/38	54/1.42	FAC	4	688	21 days	119.8	120.7	0	Not detected
38	Non-Hodgkin lymphoma	F/42	43/1.31	R-CHOP	5	1000	21 days	90.2	88.9	0.1355	Not detected
39	Breast Cancer	F/47	56/1.53	FAC	5	757	21 days	324.8	330.0	0	Not detected
40	Breast Cancer	F/42	64/1.70	FAC	5	850	29 days	408.7	385.9	2.4075	Detected
41	Non-Hodgkin lymphoma	M/34	59/1.629	C+Rituximab. 5-fluorourasil	4	400	29 days	144.1	140.3	0	Not detected
42	Non-Hodgkin lymphoma	M/43	64/2.00	C+Vincristine. doxorubicin	6	1300	21 days	191.9	193.6	0	Not detected
43	Breast Cancer	F/39	65/1.54	FAC	4	770	21 days	95.1	97.0	0	Not detected
44	Breast Cancer	F/51	45.5/1.50	FAC	4	770	21 days	144.6	145.4	1.5094	Detected
45	Breast Cancer	F/55	68/1.62	FAC	4	810	21 days	239.5	240.3	0	Not detected
46	Non-Hodgkin lymphoma	M/58	71/1.80	C+Vincristine. doxorubicin	6	1350	21 days	356.1	357.0	0	Not detected
47	Breast Cancer	F/44	56/1.53	C-docetaxel	4	760	21 days	338.4	339.5	0	Not detected
48	Non-Hodgkin lymphoma	M/52	73/1.867	FC	4	1400	29 days	213.8	226.4	5.868	Terkuantitasi
49	Breast Cancer	F/48	52/1.48	FAC	5	750	21 days	534.6	535.1	0	Not detected
50	Breast Cancer	F/46	69/1.635	CMF	5	981	21 days	148.2	149.1	0	Not detected
51	Breast Cancer	F/53	78/1.803	FAC	5	844	21 days	168.9	179.5	0	Not detected
52	Breast Cancer	F/61	69/1.78	FAC	5	805	21 days	88.4	89.4	0	Not detected
53	Breast Cancer	F/49	61/1.62	CMF	5	800	29 days	161.0	159.4	0	Not detected

54	Breast Cancer	F/49	36/1.43	FAC	5	800	29 days	206.9	210.2	1.9199	Detected
55	Breast Cancer	F/58	52/1.49	C-docetaxel	6	900	21 days	358.1	358.3	3.0160	Detected
56	Breast Cancer	F/52	63/1.65	CMF	5	970	21 days	105.1	107.2	1.1451	Detected
57	Breast Cancer	F/32	57/1.5	FAC	6	766	21 days	269.8	242.9	0.1312	Not detected
58	Breast Cancer	F/56	65/1.61	AC	4	960	21 days	472.2	473.5	2.0337	Detected
59	Breast Cancer	F/56	63/1.63	CMF	6	976	21 days	115.5	115.4	0	Not detected
60	Breast Cancer	F/40	63/1.655	FAC	6	781	21 days	250.1	250.6	0.0546	Not detected
61	Breast Cancer	F/57	44/1.217	C-docetaxel	6	730	29 days	39.9	-	3.4388	Detected
62	Breast Cancer	F/50	50/1.48	CMF	6	896	21 days	145.9	146.1	3.1965	Detected
63	Non-Hodgkin lymphoma	M/56	51/1.482	RCOP + epirubicin	6	1200	21 days	115.9	117.0	0.4594	Not detected
64	Non-Hodgkin lymphoma	F/49	71/1.720	R-CHOP	6	1300	21 days	133.3	162.4	1.0954	Detected
65	Breast Cancer	F/48	89/2.00	C-docetaxel	6	982	21 days	127.7	128.6	0.1649	Not detected
66	Non-Hodgkin lymphoma	M/32	54/1.467	R-CHOP	4	1100	21 days	707.7	682.7	0	Not detected
67	Breast Cancer	F/60	65/1.433	C-docetaxel	4	800	21 days	29.0	29.4	1.6049	Detected
68	Breast Cancer	F/51	63/1.45	C-docetaxel	4	820	21 days	177.3	200.1	3.2589	Detected
69	Breast Cancer	F/58	50/1.47	FAC	6	690	21 days	238.6	241.0	1.2521	Detected
70	Non-Hodgkin lymphoma	F/67	69/2.13	RCOP + epirubicin	4	1600	21 days	148.8	149.4	1.4081	Detected
71	Breast Cancer	F/52	68/1.58	CMF	4	953	21 days	242.2	217.9	1.3940	Detected
72	Non-Hodgkin lymphoma	F/42	45/1.33	R-CHOP	5	1000	21 days	153.9	152.1	0.4175	Not detected

9) The fragmentation was predicted by the author. by correlating the loss of mass with the loss of the group.

Ref.13 has been corrected in accordance with the referee's commentary.

✓ Is O⁶-methylguanine stable in formic acid (90 % v/v) at 80°C for 1 hour?

Is there the possibility that a quantity of the analyte is lost by the effect of the acid (the authors used 90 % v/v formic acid) and temperature? According to the authors: "The DNA solution (200 µL) was mixed with HPLC grade water and formic acid (90%) in equal volume. The solution was mixed and heated at 80 °C for 60 minutes using a Thermomixer (Eppendorf) [19]. The resulting hydrolyte was injected into the UPLC-MS/MS."

We have added a reference for this [22] Zhang. F., Bartels. M.J., Pottenger. L.H., Gollapudi. B.B. & Schisler. M.R. (2006). Simultaneous quantitation of 7-methyl- and O6-methylguanine adducts in DNA by liquid chromatography-positive electrospray tandem mass spectrometry. J. Chromatogram. B. 833. 141-148

✓ How many replicates did the authors perform? Duplo (2 replicates)