Signs of late radiation-induced genomic instability in persons chronically exposed to radiation.

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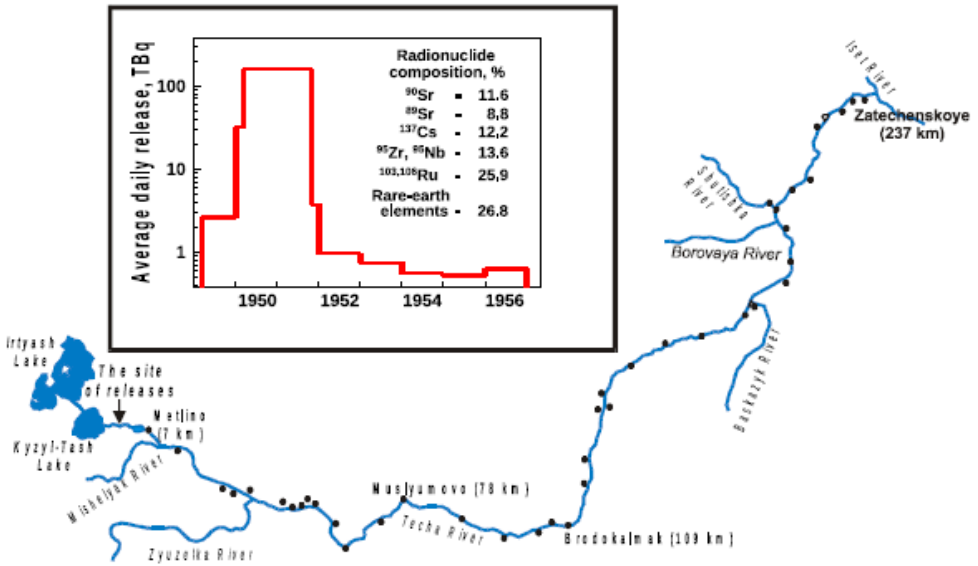
IRPA13, Glasgow, UC, 2012

Techa River Cohort

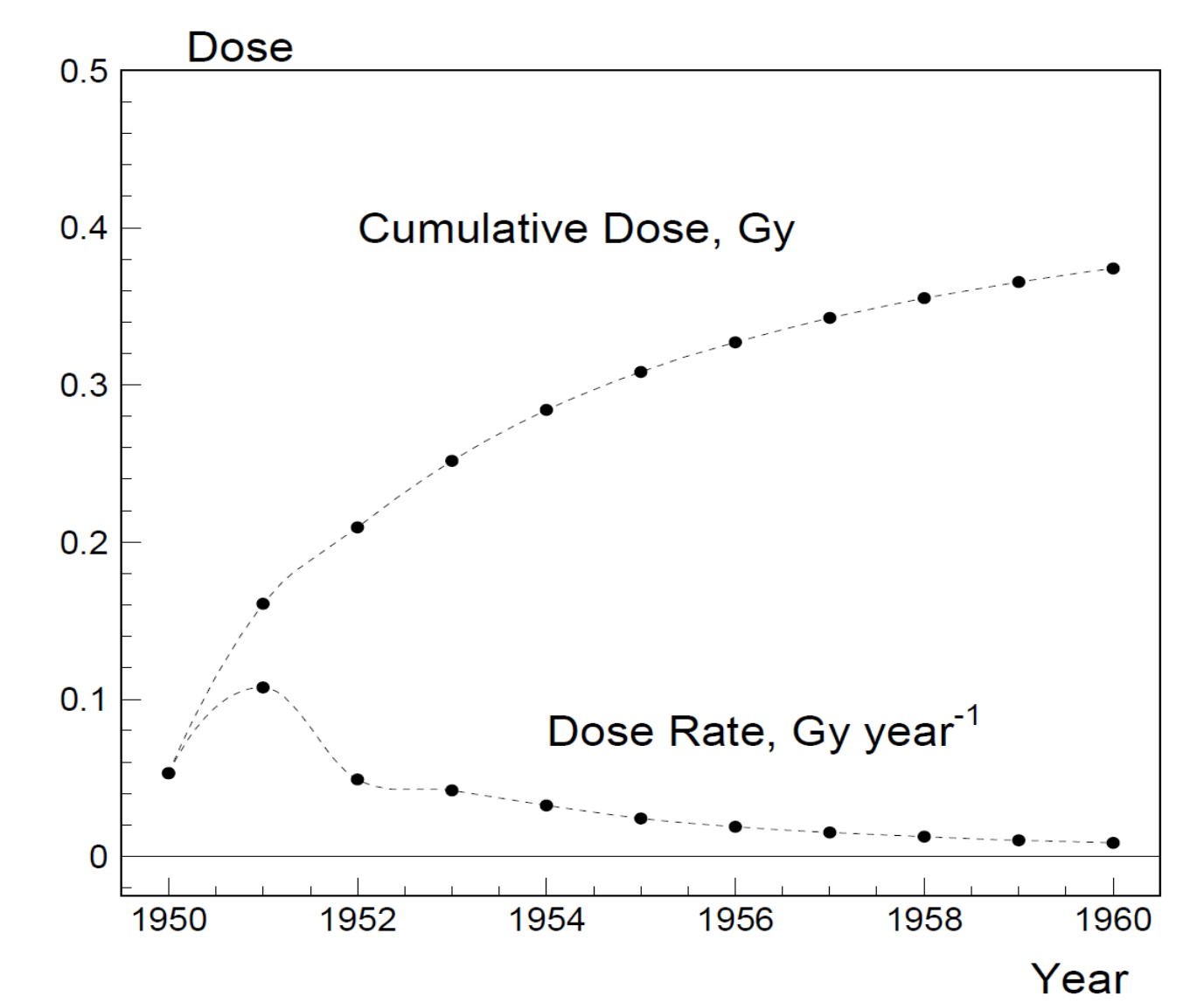
Location of Catchment Area

Techa Rover (Mean annual releases into the Techa)



- Extended Techa River Cohort (ETRC) includes about 30,000 individuals exposed to protracted IR as a consequence of released radioactive waste into the Techa River during the initial years (1949-1956) of operation of the Mayak nuclear facility (weapon grade plutonium production) in the Southern Urals region of Russia near Chelyabinsk city *(Kossenko et al., 2005, Radiat. Res., 164(5): 591-601.)*.
- The maximum cumulative doses to red bone marrow (RBM) reached 6 Gy and were mostly accumulated during the early years of exposure (i.e., 1949-1956).
- ETRC (as well as the cohort of offspring) represent “natural” unselected population.

Dynamics of dose rate and cumulative dose (RBM)



Research Methods

The following variables were considered:

- CD3-CD4+lymphocytes;
- Dicentrics;
- Micronucleus;
- Mutations in Tp53;
- DNA double-stranded breaks / DNA single-stranded breaks;
- Double-stranded break IRC / Single-stranded break IRC,
- Cu/Zn SOD, Nitrate (NO3-), Nitrite (NO2-);
- Apoptosis;
- *Chk2 and Ki-67.*

Background and Research hypotheses

Background

1.There is an excess in risk of leukemia in Techa River Cohort
2.It is well-known that any cancer is underlied by genetic and epigenetic changes (damages).

Research hypotheses

1.Frequencies of genetic anomalies are increased in TRC at late time after onset of exposure
2.Frequencies of genetic anomalies are increased due to inefficient operation of intracellular barrier mechanisms
3.There exists associations between frequencies of genetic anomalies and radiation dose at late time after onset of exposure

Leukemia

Excess of Relative Risk per Gy

Total	**4.2 (95% CI 1.2; 13)**	**P < 0.001**
without CLL	**6.5 (95% CI 1.8; 24)**	**P < 0.001**

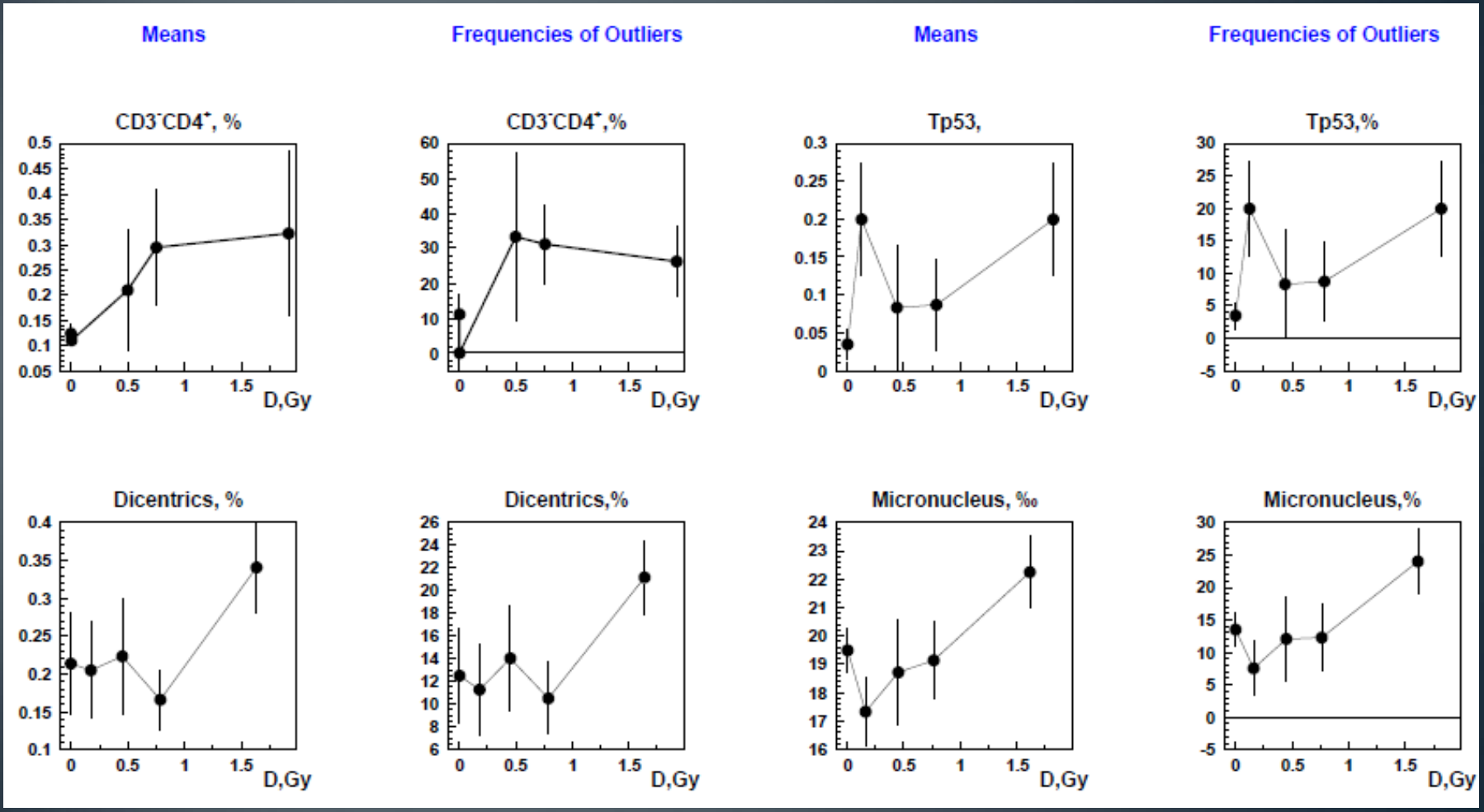
Krestinina L., Yu D.L., Preston E.V., Ostroumova et al., 2005 Radiat.Res., 164(5): 602-611.

Incidence of genetic pathology

Parameter	M±m, N				Ref	N of subjects with parameter values above the Ref			
	Exposed.	N	control	N		Exposed		Control	
						n	%	n	%
CD3-CD4+ lymphocytes	0.29±0.09 p=0.07	40	0.12±0.02	54	0.24	11 p=0.04	44.0	6	5.9
dicentrics, %	0.26±0.03	375	0.21±0.07	64	0.75	59	15.7	8	12.5
Micronucleus, $\%_0$	19.9±0.7	177	19.5±0.8	178	29.7	28	15.8	24	13.5
Mutations in Tp53	--*	101	--	73	--	18 p=0.001	17.8	1	1.4

Note: *discrete values, p: significance of differences versus the respective values in the control

Genetic abnormalities: dependence on RBM exposure dose



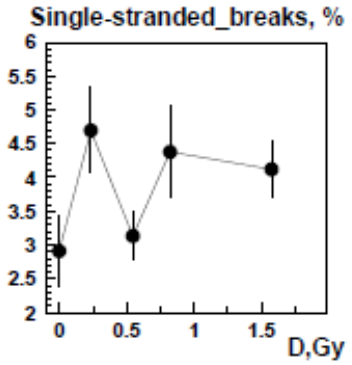
DNA single- and double-stranded breaks frequency and repair effectiveness

Parameter	M±m, N				Ref	N of subjects with parameter values above Ref			
	Exposed	N	Control	N		Exposed		Control	
						n	%	n	%
DNA double-stranded breaks, %	17.9±1.4	14	9.9±1.3	16	15.0	10 p=0.001	71.4	2	12.5
DNA single-stranded breaks, %	5.6±0.5	13	3.2±0.6	16	5.7	9 p=0.002	69.2	2	12.5
Double-stranded break IRC	67.1±3.4 p=0.006	13	48.1±4.6	13	64.6	6	46.2	2	15.4
Single-stranded break IRC	22.7±1.6	10	20.9±2.5	13	29.7	1 p=0.1	10	3	23.1

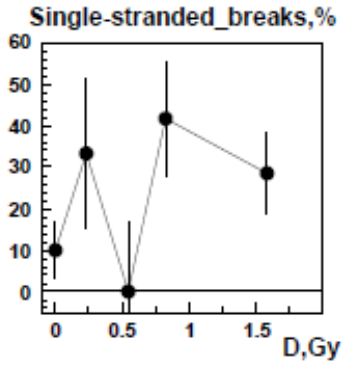
Note: IRC - ineffective repair criterion

Frequency of DNA single- and double-stranded breaks and repair effectiveness; dependence on RBM exposure dose

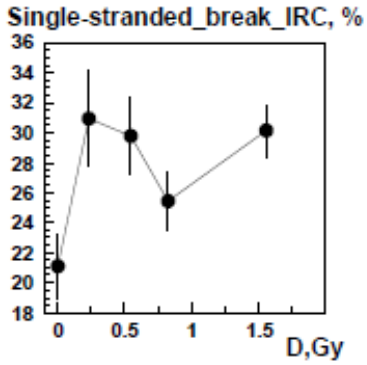
Means



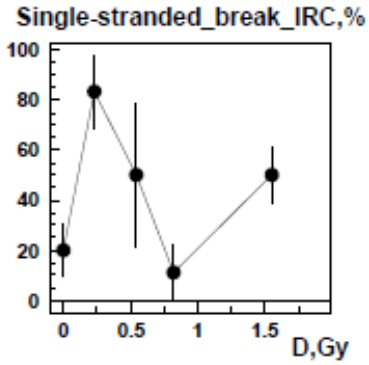
Frequencies of Outliers

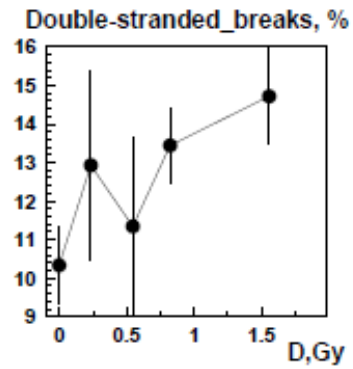


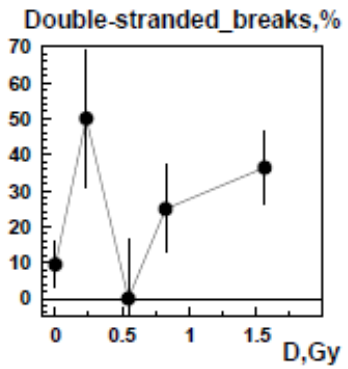
Means

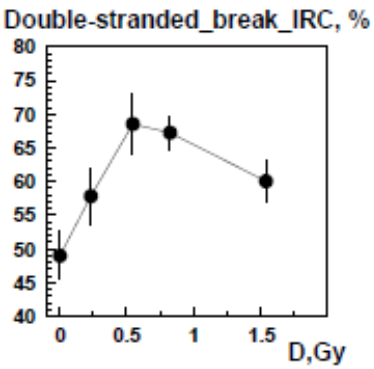


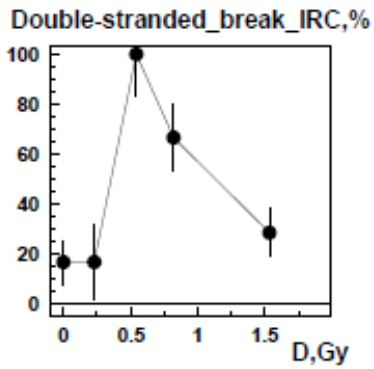
Frequencies of Outliers









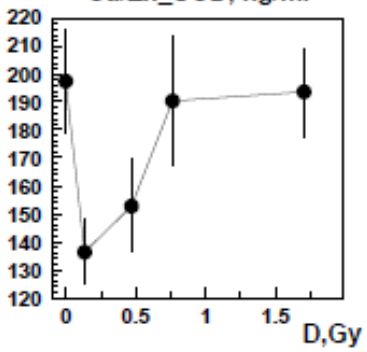


Parameters of oxidative processes

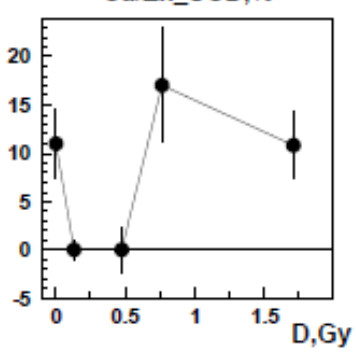
Parameter	M±m, N				Ref	N of subjects with parameter values above Ref			
	Exposed	N	control	N		Exposed		Contr	
						n	%	n	%
Cu/Zn SOD, ng/ml	174.2±9.1	193	197.3±18.7	73	357.7	16	8.3	8	11.0
MDA, µmol/l	42.0±0.8 p=0.1	165	44.6±1.5	61	56.4	16 p=0.02	9.7	13	21.3
Nitrate (NO_3^-), µmol/l	19.8±1.6 p=0.002	83	28.6±2.2	49	43.7	3 p=0.03	3.6	7	14.3
Nitrite (NO_2^-), µmol/l	26.6±1.7 p=0.002	83	18.0±2.1	50	32.9	41 p=0.02	49.4	14	28.0

Parameters of oxidation processes. Dependence on RBM exposure dose

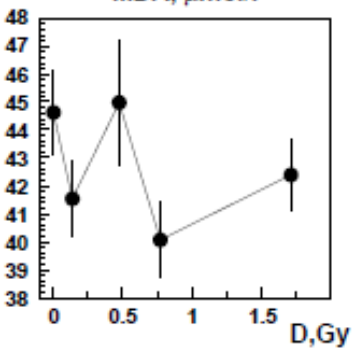
Means



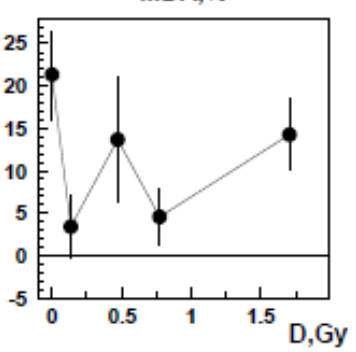
Frequencies of Outliers

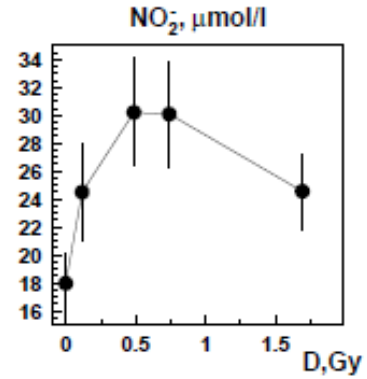


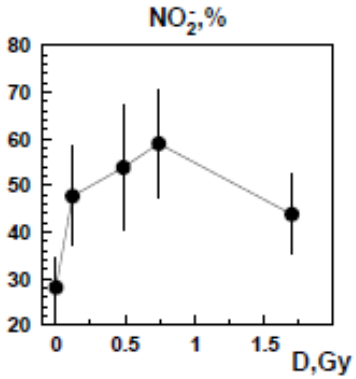
Means

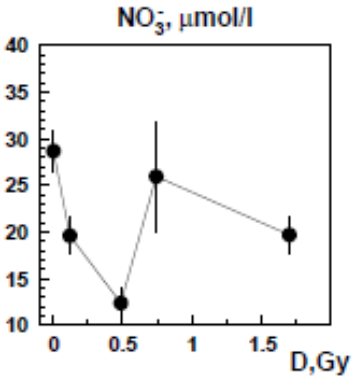


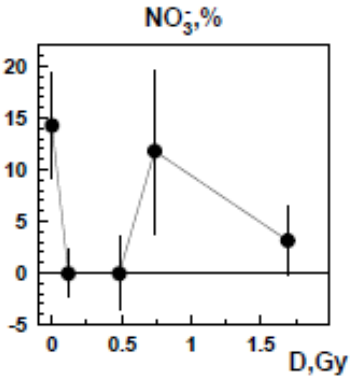
Frequencies of Outliers







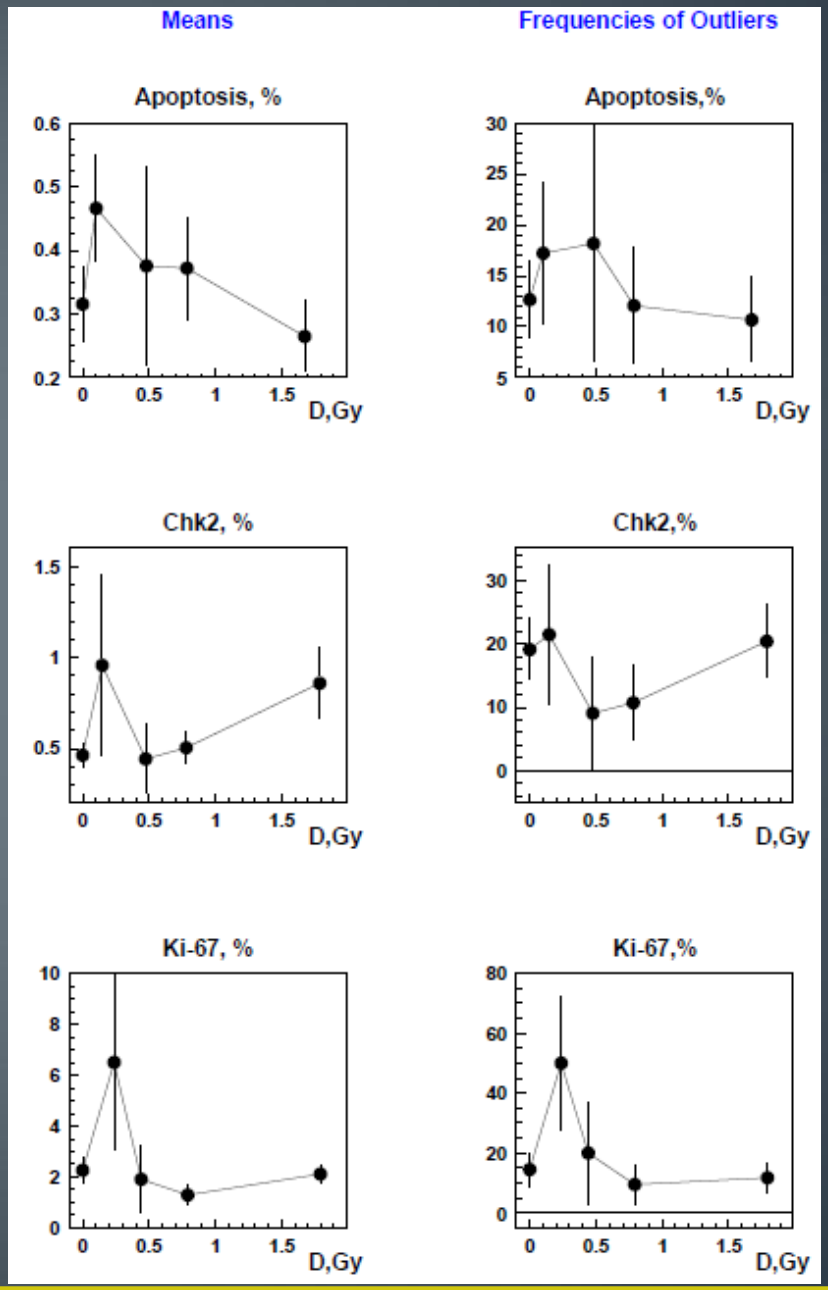




Characteristics of functional processes in cells

Parameter	M±m, N				Ref	N of subjects with parameter values above Ref			
	Exposed	N	Control	N		Exposed		Control	
						n	%	n	%
Apoptosis , %	0.4±0.04	129	0.3±0.1	79	0.8	17	13.2	10	12.7
Chk2, %	0.7±0.1 p=0.05	102	0.5±0.1	68	1.0	17	16.7	13	19.1
Ki-67, %	2.1±0.3	72	2.3±0.5	41	5.3	10	13.9	6	14.6

Parameters of functional processes in cells. Dose-dependence.



Conclusion

At late time after the onset of chronic radiation exposure an increased rate of genetic disorders was noted among residents of the Techa riverside villages.

The increased frequency of mutations/aberrations is, evidently, related to genomic instability which developed as a late response to radiation exposure. The phenomenon is associated with ineffective functioning of such intracellular mechanisms as antioxidant system, repair of DNA breaks, cell-cycle control and apoptosis.

The dose dependence of the changes observed is determined by an increase in the number of subjects with excess reference value rather than by deviations of individual values in most members of the dose sub-group. Evidently, genomic instability has developed in a small proportion of subjects who should be assigned to the group at high risk of cancer development. The proportion depends on radiation dose.

Conclusion

The patterns of dose-dependence for mean values in the subgroups are determined by the type of the parameter studied:

The values of parameters reflecting pathological processes increase with dose and reach the maximum at doses above 1.0 Gy.

The values of the parameters reflecting cell's functional status (except for the antioxidant system) at small doses (up to 0.3 Gy) are higher than the respective values obtained for unexposed persons, while at higher doses the parameter values are diminishing.

The oxidation status is characterized by a sharp reduction in the concentration of Cu/ZnSOD, already at doses below 0.3 Gy, and by re-distribution of free active species for the benefit of their most aggressive forms.

REPOSITORY OF DNA AND BLOOD SAMPLES FOR RESIDENTS OF THE TECHA RIVERSIDE VILLAGES

The tissue bank available at the URCRM is intended for preservation of the unique biological material donated by members of the Techa riverside communities: Techa cohort members and their offspring. Currently, the bank of DNA and blood samples is well-established, and it is continuously augmented. Stored at the repository, are blood samples of 3,262 donors from the following population groups:

- Techa River Cohort: 2,371 persons (1,697 women and 674 men)
- Offspring cohort (F1): 497 persons (329 women, 168 men).
- Unexposed controls: 394 persons (310 women, 84 men)
- Family units (father, mother, F1offspring): 150 (96 family units from the TRC, 54 family units from the unexposed controls).

Data available on each donor: gender, ethnicity, age at sample collection, date of sample collection, dosimetric characteristics, medical diagnoses.

Blood samples are stored in low-temperature refrigerators at 85 ^{o}C in 5ml test tubes with EDTA. On the average, 3 test tubes are available on each donor (15 ml of blood).

DNA was extracted by the phenol-chloroform extraction method, without proteinase, using a task-oriented approach. DNA was extracted in 50 persons.

Thank you for your attention

Church and Mill, Metlino, 1965 (with gratitude to N.G.Safronova)

Church and Mill, Metlino, 1997