Meta-analysis of cancer triploidy: Rearrangements of genome complements in male human tumours are characterised by XXY karyotypes

Ninel M Vainshelbaum [1,2]**, Pawel Zayakin** [1]**, Regina Kleina** [3]**, Alessandro Giuliani** [4]**, and Jekaterina Erenpreisa** [1,*]

1   Latvian Biomedical Research and Study Centre, Riga, LV-1067, Latvia; katrina@biomed.lu.lv
2   The University of Latvia, Riga, LV-1586, Latvia; ninela.vainselbauma@biomed.lu.lv
3   Riga Stradins University, Riga, LV-1007, Latvia; rkleina@inbox.lv
4   Istituto Superiore di Sanità, Rome, 00161, Italy; alessandro.giuliani@iss.it
*   Correspondence: katrina@biomed.lu.lv;

Abstract: Triploidy in cancer is associated with poor prognosis but its origins remain unclear. Here, we attempted to differentiate between random chromosomal and whole-genome origins of cancer triploidy. *In silico* meta-analysis was performed on 15 male malignant and 5 benign tumour cohorts (2928 karyotypes) extracted from the Mitelman Database, comparing their ploidy and combinations of sex chromosomes. A distinct near-triploid fraction was observed in all malignant tumour types, being especially high in seminoma. For all tumour types, X-chromosome doubling, predominantly observed as XXY, correlated strongly with the near-triploid state (r≈0.9, p<0.001), negatively correlated with near-diploidy, and did not correlate with near-tetraploidy. A smaller near-triploid component with a doubled X-chromosome was also present in 3 of 5 benign tumour types, especially notable in colon adenoma. Principal Component Analysis revealed a non-random correlation structure shaping the X-chromosome disomy distribution across all tumour types. We suggest that doubling of the maternal genome followed by pedogamic fusion with a paternal genome (a possible mimic of the fertilization aberration, 69, XXY digyny) associated with meiotic reprogramming may be responsible for the observed rearrangements of genome complements leading to cancer triploidy. The relatively frequent loss of the Y-chromosome results secondary from chromosome instability.

Keywords: cancer near-triploidy, male tumours, karyotype meta-analysis, XXY, whole genome rearrangements, digyny.

1. Introduction

Aneuploidy (an abnormal number of chromosomes) is a well-known hallmark of malignant tumours, generally associated with their aggressive development [1,2]. With results of cancer genome sequencing projects revealing flaws in the mutation theory, inability to explain the chemoresistance, and for providing targeted therapies, in general, with frustrating clinical benefit, the aneuploidy theory of cancer proposed in the 19th century by David Hansemann and Theodor Boveri, is enjoying its renaissance [3]. The aneuploidy theory posits the whole-genome chromosome instability (CIN) as causally responsible for the propagation of cancer. Currently, CIN is viewed as a system behavior of a stress response with adaptive advantages of microevolution (including Darwinian clonal selection) which also serves as a new potential cause of further destabilization of the genome that leads to genome crisis (chromothripsis), which results in a rapid and massive genome reorganization (punctuated evolution) unifying the diverse chromosomal and nuclear abnormalities [4,5]. Recent advances in the molecular characterisation of

aneuploidy revealed that the search for the general mechanism of how aneuploidy contributes to cancer is becoming increasingly challenging: it appears that aneuploidy can be linked to diverse molecular pathways [6] and favours both tumour suppressing and driving effects [7,8]. One more cloud is coming over the victory march of chaotic adaptations in cancer evolution. This is the so-called "Muller's ratchet" (Muller 1964). In his works, H.Muller postulated that neutral and harmful mutations (also inevitably caused by structural chromosome imbalances) should sooner or later lead to the extinction of the asexual species. However such unicellular species, e.g. lobose amoebas, have existed on Earth for two billion years. The same relates to somatic tumours of the mammals - like protists, they are immortal and we still do not have an answer, why? Interestingly, many agamic protists undergo cyclic polyploidy [9,10]. In turn, polyploidy and aneuploidy in human cancer cells are associated with genome reprogramming [3,11]. Moreover, chromothripsis which is crushing the chromosome order should cause de-speciation of mammalian tumour cells. Indeed, the atavistic recapitulation in human tumour cells of the unicellular programs is becoming apparent [12–14]. Interestingly, also this epigenetic shift is associated with polyploidy [15–17].

It appears that an exit from the obstacle of the Muller's ratchet may be provided by the paradoxical option for the aneupolyploid genome to convert chaos into order using the reprogramming and rearrangements of whole genomes acting in ploidy cycles. As operating with the genome complements, those differ from the mitotic cycles, whose task is to orderly segregate chromosomes. The reproductive ploidy (life) cycles are orderly doubling and halving the whole genome complements with the help of meiosis, performing recombination, reduction, and also gene conversion [18,19]. The elements of this meiosis-like mechanism are likely implemented in the asexual or parasexual "life-cycles" of reprogrammed cancer cells, which are reciprocally joined with mitotic cycles [20–23].

Among aneuploidies of solid tumours, a near-triploid karyotype is often a hallmark of chemotherapy resistance and thus of the increase of survival potential [24,25]. We paid attention to the fact that among the numerical sex chromosome aberrations in the tumours of male patients, the assertive acquisition of an extra X chromosome and frequent loss of the Y chromosome have been reported in several cases [26–32], particularly in association with triploidy in the male germ cell tumour seminoma [33].

Here, we decided to use the advantage of the presence of two different sex chromosomes, X and Y, in a normal diploid male karyotype, in order to attempt the differentiation between two potential constituents of tumour near-triploidy - the chromosomal aberrations and the rearrangements of the whole-genome complements. For this purpose, we performed an *in silico* meta-analysis of the male tumour karyotypes deposited in the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer [34].

2. Materials and Methods

The karyotypes from 15 male malignant solid tumour types (untreated and presented in the >50 number of cases), epithelial and mesenchymal, somatic and germinative, and karyotypes from 5 benign tumour types were obtained from the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer [34] in the period of January-March, 2019. None of the male patient karyotypes were affected with congenital sex chromosome aberrations such as Kleinfelter syndrome. The types of tumours and the number of patient karyotypes for each of them are presented in Table.1.

Table 1. The analyzed male tumour types, the number of karyotypes per cohort, the percent share of near-triploidy (in the range 62-76 chromosomes), and the percent share of sex chromosome configurations containing a disomic X-

chromosome. The karyotype XXY in most cases indicates near-triploidy with the three sex chromosome complement XXY. XX,-Y largely means a near-triploid karyotype from a male with loss of chromosome Y, where "-Y" indicates the third haploid chromosome set. Sex chromosomes XY,+X indicate a near-diploid male karyotype with (not inherited) acquisition of the extra sex chromosome X. The diploid (or near-diploid) male karyotype X,-Y,+X means (acquired) loss of sex chromosome Y and gain of sex chromosome X, while karyotype XXY,+Y is near-triploid by chromosome number, with a gain of chromosome Y.

№	Malignant tumour type	Number of karyotypes	% of near-triploidy (62-76)	XXY %	XX,-Y %	(XY,+X)+ (X,-Y,+X) %	XXY, +Y %
1	Seminoma	78	42.31	47.44	3.85	5.13	10.26
2	Osteosarcoma	61	27.87	24.59	3.28	0.00	6.56
3	Lung carcinoma	237	27.00	8.02	9.70	2.53	2.53
4	Gastric carcinoma	74	20.27	10.81	5.41	6.76	1.35
5	Head and neck squamous cell carcinoma	191	16.75	5.76	8.90	0.52	1.57
6	Colon adenocarcinoma	98	16.33	12.24	6.12	10.20	6.12
7	Transitional cell carcinoma	104	13.46	4.81	3.85	1.92	1.92
8	Chondrosarcoma	85	11.76	4.71	3.53	3.53	0.00
9	Malignant melanoma	134	10.45	5.22	3.73	2.24	2.24
10	Glioblastoma	215	10.23	7.44	1.40	0.00	1.40
11	Renal carcinoma	577	7.11	3.81	4.68	1.04	1.21
12	Mesothelioma	72	6.94	5.56	2.78	0.00	2.78
13	Rhabdomyosarcoma	92	6.52	3.26	1.09	3.26	0.00
14	Ewing sarcoma	228	3.51	3.95	0.00	3.51	0.88
15	Liposarcoma	147	3.40	1.36	1.36	0.00	0.00
	Benign tumour type						
16	Colon adenoma	62	11.29	11.29	4.84	0.00	0.00
17	Astrocytoma	59	6.78	1.69	1.69	1.69	1.69
18	Lipoma	235	0.85	0.85	0.00	0.00	0.43
19	Renal adenoma and oncocytoma	48	0.00	0.00	2.08	0.00	0.00
20	Salivary gland adenoma	131	0.00	0.00	0.76	0.00	0.00

The tumour Nomenclature used was based on the International Classification of Diseases for Oncology (ICD-O), the Systematized Nomenclature of Medicine (SNOMED), and the WHO Classification of Tumours of Soft Tissue and Bone - the same sources as the Mitelman database's nomenclature. Seminoma was the germ cell tumour. Among somatic tumours, the lung carcinoma cohort included a total of 5 lung tumour types (squamous cell carcinoma, adenosquamous carcinoma, adenocarcinoma, undifferentiated large cell carcinoma, and small cell carcinoma), united from the evidence that both bronchoepithelial and neuroendocrine lung stem cells likely have one common precursor [35]. The gastric carcinoma cohort was comprised of adenocarcinoma and undifferentiated carcinoma. These cases were not sorted by stages of the malignant process in the Mitelman database. Only monoclonal karyotypes comprising in total 2928 tumour cases were collected, filtering out

the cases with polyclonal karyotypes, the cases where several samples were obtained from one patient, and incomplete sex chromosome karyotypes. Using the data analysis tools of the numpy [36], pandas [37] and scipy [38] Python libraries, statistical analysis of the available data was performed to determine the relationship between modal chromosome numbers and different sex chromosome karyotypes were analysed.

The 2013 edition of the International System for Human Cytogenetic Nomenclature (ISCN) defines near-triploidy as a modal chromosome number that falls in the 58-80 range [39]. In our study, the boundaries of triploidy were also narrowed to a medium-sized range spanning 62-76 chromosomes, and a narrower range spanning 66 to 72 chromosomes. The nomenclature of the sex chromosome karyotypes was used the same as presented in the Mitelman database using ISCN, where a sex chromosome complement is expressed as related to ploidy level.

Principal Component Analysis (PCA) was applied to the X-chromosome disomy (#X-disomy) distribution across different tumour types in order to check for the departure of the X-chromosome (#X)-disomy patterns from randomness [40]. The departure from the randomness of such solution was estimated by means of Bartlett corrected chi-square as applied to maximum-likelihood factor extraction [41].

3. Results

3.1. Analysis of the histograms of the modal chromosome numbers in 15 cohorts of malignant tumours

In all examined malignant solid tumour types, listed in Table 1, the aneuploid karyotypes were present. The summary histograms of the modal chromosome numbers of each cohort are presented on Fig.1.

It is seen that they include near-diploid karyotypes, near-triploid karyotypes, a degree of tetraploidy (high in rhabdomyosarcoma) and in some cases also hyper-tetraploid karyotypes. The near-triploid karyotypes were present in all malignant tumour types. Their percentual share for malignant tumour types 1-15 is presented in Table 1 in the descending order. In particular, the high proportion of near-triploidy (42%) was observed for the germ tumour, seminoma. In 14 examined somatic malignant tumour types, both epithelial and mesenchymal, the near-diploid karyotypes were dominating, while the proportion of near-triploid ones was less pronounced than in seminoma, albeit in a varying degree (Table 1, Fig.1). Osteosarcoma was a leader in triploidy (28%). Lung carcinoma also displayed a high proportion of near-triploid karyotypes (27%), other somatic tumours showed lower values.

3.2. Analysis of the sex chromosome sets with #X-disomy in each malignant tumour cohort in relation to ploidy of their karyotypes.

We also analysed the sex chromosome sets with #X-disomy, which are presented alongside their percentage for each malignant tumour cohort in Table 1. It can be seen that configuration XXY dominates in seminoma and is also predominant among #X-disomic karyotypes in 10 of 14 somatic malignant tumours. However the proportion of XX,-Y set is larger in them than in seminoma, while in head and neck (HN) squamous cell carcinoma XX,-Y is prevailing over XXY. Other karyotypes with #X-disomy (XY,+X) + (X,-Y,+X) were a minority, with the exception of colon adenocarcinoma, where their proportion was comparatively high. Some of the (largely near-triploid) XXY karyotypes were also revealed to possess an extra Y chromosome (especially evident in seminoma, osteosarcoma and colon adenocarcinoma); their percentual share is presented in Table 1.
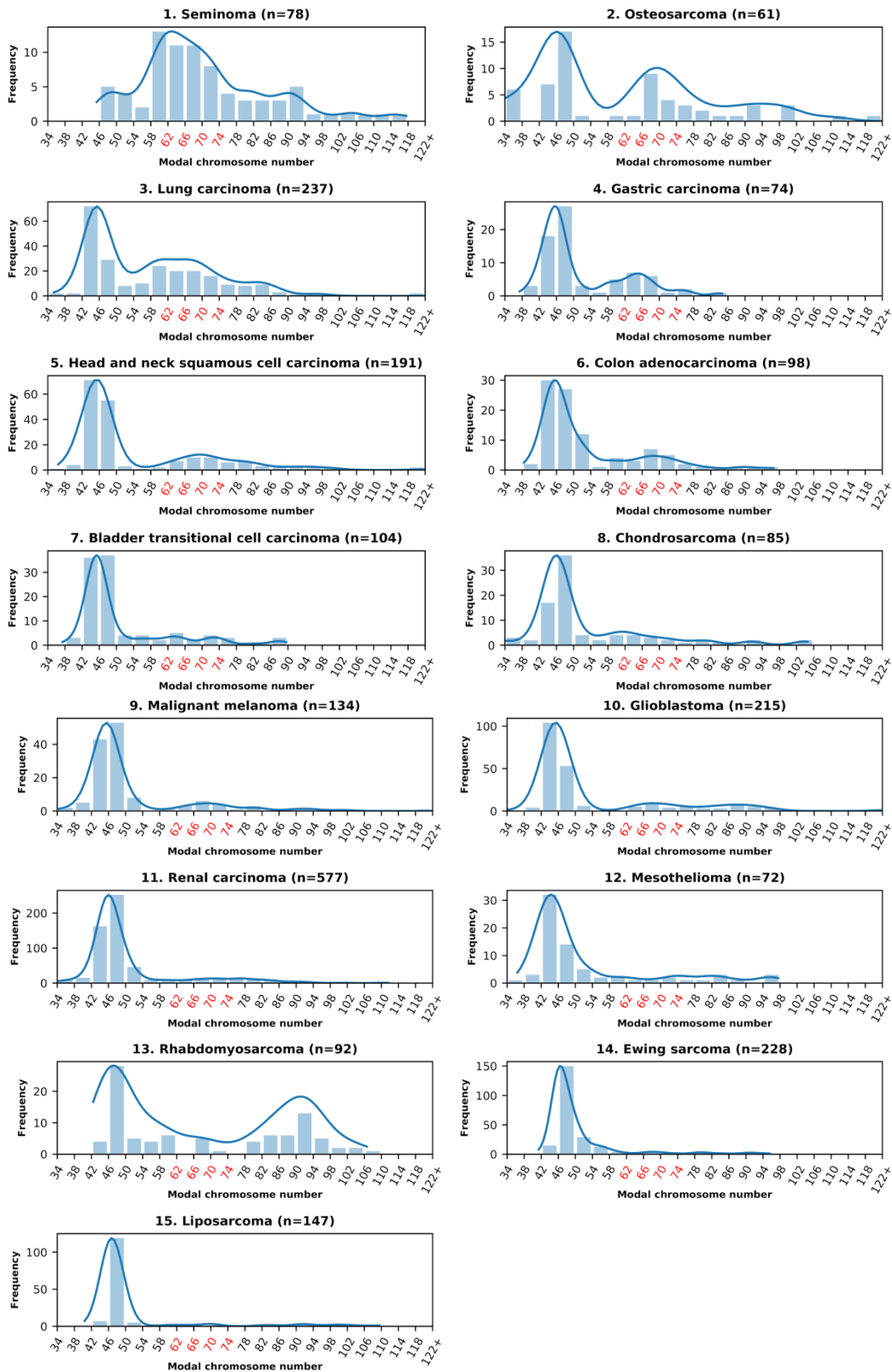
Figure 1. The modal chromosome number frequency histograms of 15 malignant tumour cohorts, numbered as listed in Table 1. The chromosome numbers within the (arbitrarily chosen) wide range of near-triploidy (62-76 chromosomes) are marked red.

Further, we compared the relationship of the karyotypes exhibiting #X-disomy with different ploidy ranges of the modal chromosome numbers for all malignant tumours and

for only somatic tumour cohorts, excluding seminoma. The results of this comparative statistical analysis are shown on Fig.2.
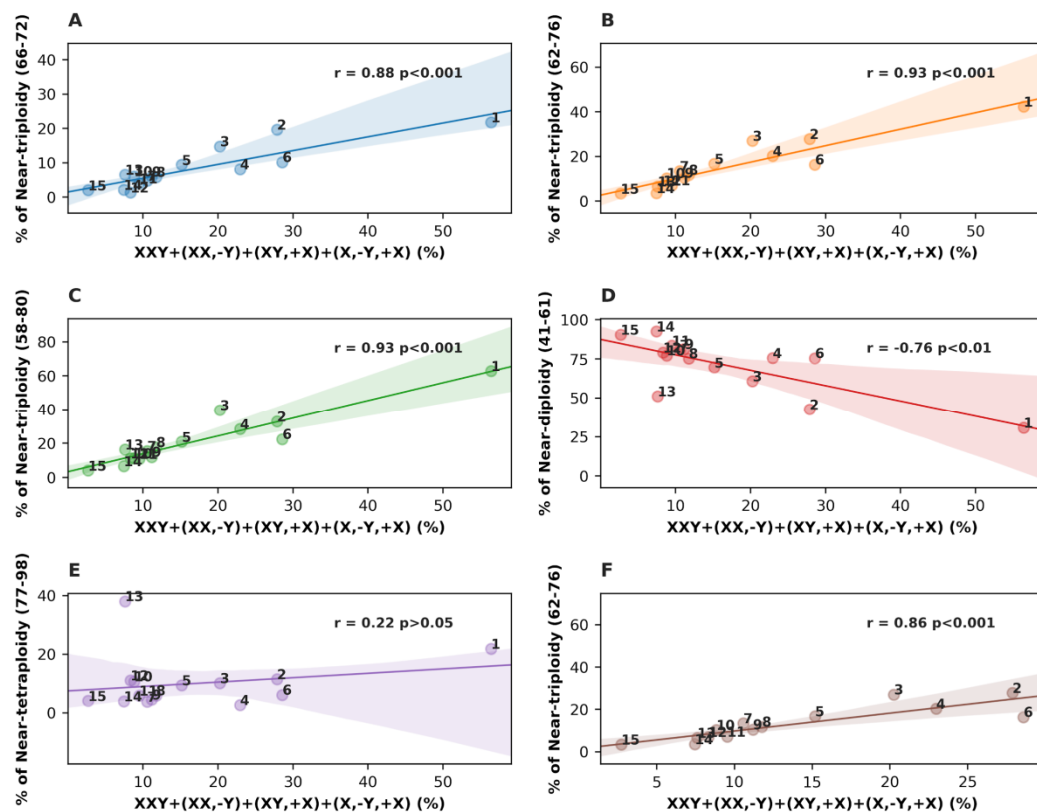
Figure 2. Results of the Pearson correlation analysis for all 15 patient karyotype cohorts of malignant tumours evaluating the relationship between all karyotypes containing doubled X-chromosomes and ploidy in different chromosome ranges: (A) in relation to the narrow triploidy range (66-71 chromosomes); (B) in relation to the median triploidy range (62-76 chromosomes); (C) in relation to the wide (ISCN) triploidy range (58-80 chromosomes); (D) in relation to the near-diploidy range (41-61 chromosomes); (E) in relation to near-tetraploidy range (77-98 chromosomes); (F) only malignant somatic tumours are presented as related to the near-triploidy median range. The tumour cohort numbers are the same as in Table 1.

Strikingly, in spite of the many-fold difference in proportions of the near-triploid karyotypes among 15 malignant tumour types, all together provided a very high Pearson correlation between #X-disomic sex chromosome karyotypes and all tested ranges of near-triploidy, from the narrower range (r=0.88; p<0.001) to the median and widest range, providing equally (r=0.93, p<0.001) (Fig.2A-C). In the near-diploidy range (Fig.2D), the correlation was strongly negative (r=-0.76, p<0.01), in the near-tetraploidy range (Fig. 2E), no correlation was observed. When excluding seminoma, the Pearson correlation of somatic malignant tumour cohorts presented on Fig.2F in the median near-triploidy range was also convincingly strong (r=0.86, p<0.001).

Further, we examined the influence of different #X-disomic karyotypes on Pearson correlation in the median range of near-triploidy (62-78 chromosomes). The results are presented on Fig.3.
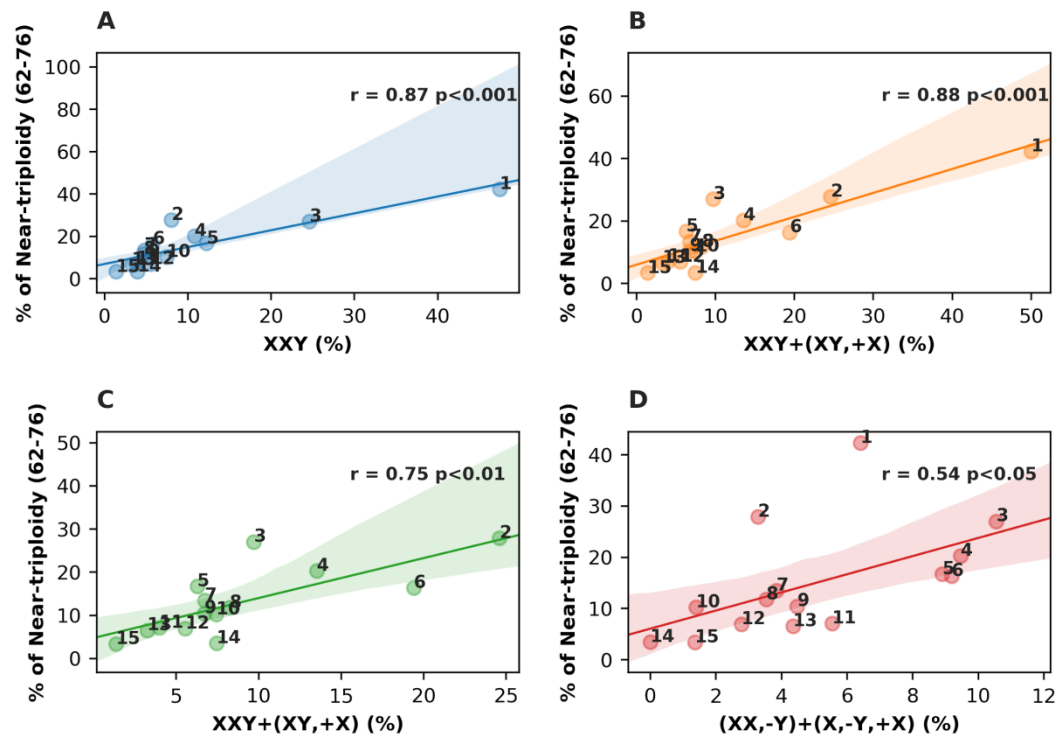
Figure 3. Results of the Pearson correlation analysis for malignant tumours evaluating the relationship between different karyotypes containing doubled X-chromosomes and ploidy in the median near-triploidy range. (A) For XXY and (B) XXY+(XY,+X) configurations; (B) all karyotypes with doubled X in relation to triploidy in the narrow range (66-72 chromosomes); (C) double X-karyotypes with an Y chromosome in relation to the wide range of near-triploidy; (D) double X-karyotypes lacking an Y chromosome in relation to the wide range of near-triploidy. The tumour cohort numbers are the same as in Table 1.

The results show a very high contribution of XXY karyotypes (Fig.3A) in near-triploidy range (r=0.88, p<0.001), which remains the same adding also near-diploid karyotypes with #X-disomy (Fig. 3B). For somatic malignant tumours only, presented on Fig.3C, this correlation with both karyotypes is a smaller but still strong (r=0.75, p<0.01). The loss of #Y from #X-disomic near-triploid and near-diploid karyotypes presented for all malignant tumours on Fig.3D weakened the correlation with near-triploidy but it still remained positive and statistically significant (r=0.54, p<0.05).

3.3. Analysis of all sex chromosome configurations in relation to near-triploidy in malignant tumours.

The relationship between near-triploidy in the wide range (62-76) and all sex chromosomes sets is presented in barplot form for all malignant tumours on Fig.4. Besides the already discussed issues of prevailing association of #X-disomy with near-triploidy, Fig.4 also reveals that a small part of XY karyotypes and X,-Y karyotypes are also near-triploid, in particular, this is pronounced in lung carcinoma. Likely, it is associated with the chromosome instability processes. Contrary to the karyotypes with doubled X-chromosome, the compositions of sex chromosomes with doubled Y and one or absent X (XYY or YY) were rare (and therefore not presented in this article), while 10 of 16 tumour types (seminoma, osteosarcoma, lung carcinoma, colon adenocarcinoma, gastric carcinoma, bladder transitional cell carcinoma, liposarcoma, chondrosarcoma, Ewing sarcoma and

glioblastoma) were lacking them. Only one near-triploid XYY - karyotype was found in the entire analyzed dataset, in rhabdomyosarcoma.
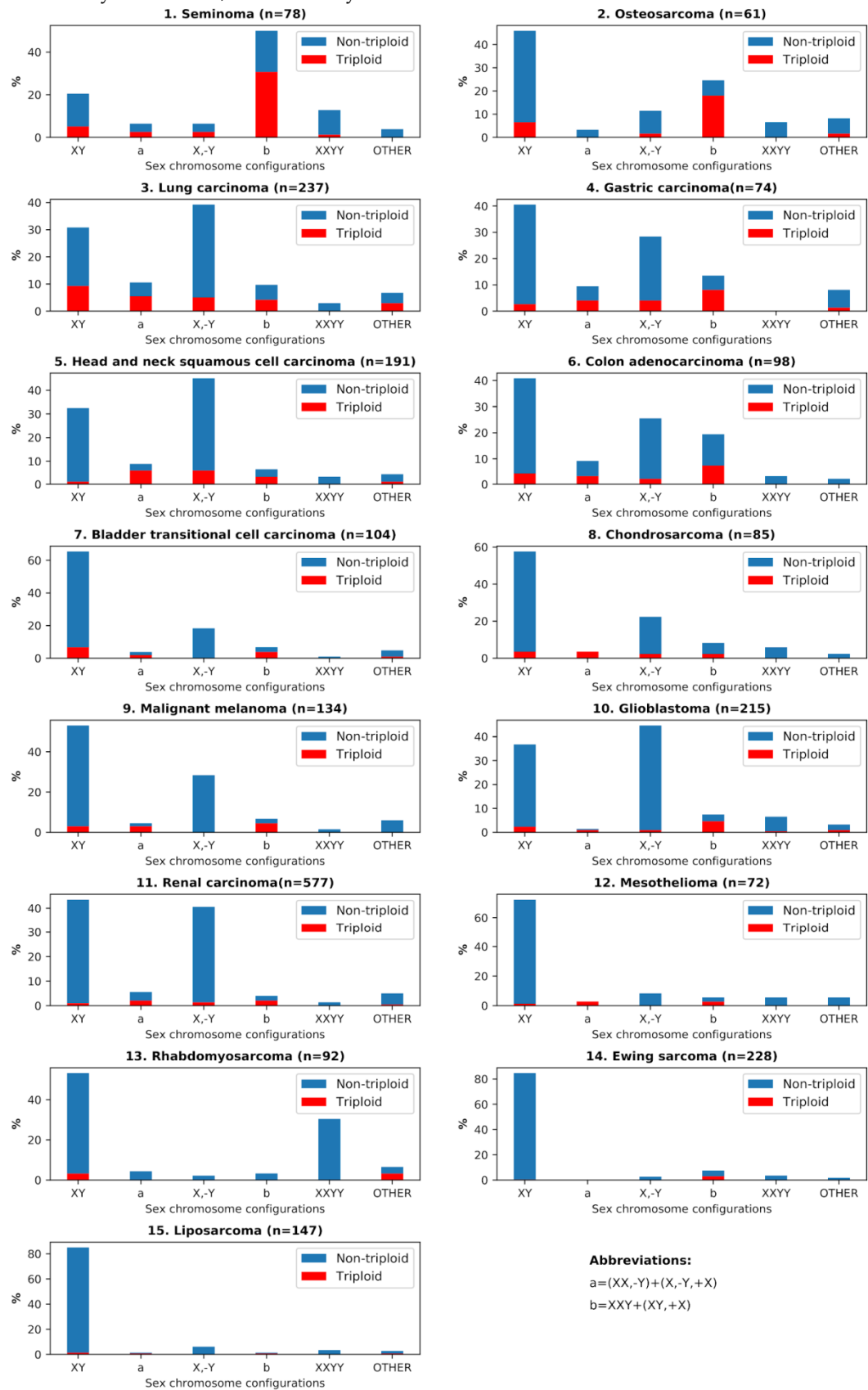
Figure 4. The percentages of different sex chromosome configurations and their respective percentages of near-triploidy (62-76 chromosomes) for all malignant tumour cohorts numbered as listed in Table 1.

3.4. Benign tumours: Study of the doubled X-chromosome karyotypes and near-triploidy.

As triploidy in association with #X-disomy was found in all malignant tumours we were interested to find out whether these features could also be observed in premalignant somatic lesions. For that four available pairs of sufficiently large tumour cohorts were compared: astrocytoma versus glioblastoma, colon adenoma versus adenocarcinoma, renal adenoma and oncocytoma versus renal carcinoma, lipoma versus liposarcoma and salivary gland adenoma added as the fifth cohort. The results are presented in Table 1 (Nr 16-20) and Fig.5.
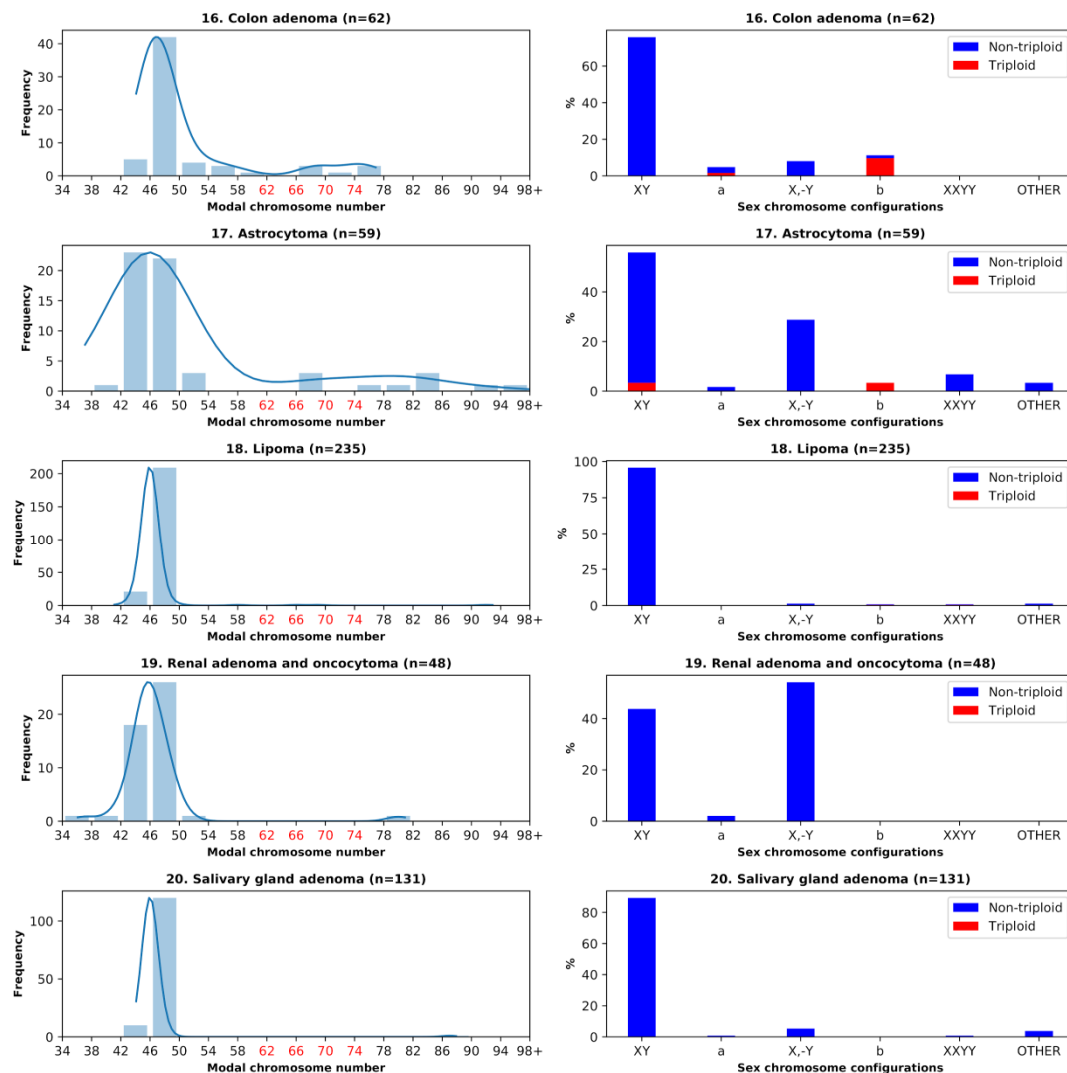
Figure 5. Left column – the histograms of the modal chromosome numbers, with near-triploidy wide range marked red. Right column - the corresponding percentages of different sex chromosome configurations with #X-disomy and their respective percentages of near-triploidy (62-76 chromosomes) for 5 benign tumour cohorts. Designations (a, b) in the right column - **a**: #X-disomic karyotypes lacking a Y chromosome (XX,-Y and X,-Y,+X); **b**: #X-disomic karyotypes with a Y chromosome (XXY and XY,+X).

In colon adenoma and adenocarcinoma, the proportion of #X-disomic karyotypes with near-triploidy was rather similarly high, however, triploidy in adenoma was lower than in colon adenocarcinoma (11% vs. 16%, respectively). All triploid colon adenoma karyotypes except one were XXY, while the remaining one was XX,-Y (Table 1, Fig.5). In astrocytoma, the total percentage of #X-disomy was more than two times lower compared to

glioblastoma, and near-triploidy was also significantly lower (6.78% to glioblastoma's 10.23%), while liposarcoma surpassed lipoma 4-fold in triploidy, and more than 3-fold in #X-disomy proportion (although both were in the lower range of the two values). The renal adenoma and oncocytoma cohort was more than 4-fold poorer with #X-disomic karyotypes than its malignant counterpart (2.08% vs. 8.49%) and did not show near-triploidy. Salivary gland adenoma also did not display near-triploidy and #X-disomy was almost absent as well (0.76%).

3.5. Principal Component Analysis (PCA)

PCA was applied for the exploration of the X-chromosome disomy (#X-disomy) pattern in order to demonstrate the non-randomness of different #X-disomy categories distribution in near-triploidy across different tumour types. The initial four-dimension space having as axes four different categories of #X-disomy, namely: #X-disomy (all types)_62-76 (chromosomes); #X-disomy (all types)_58-80; XXY+XX,-Y_ 62-76; #X-disomy (all types)_66-72 and 15 malignant tumor types as statistical units, was submitted to PCA (Table 3).

Table 3. PCA Loading Pattern.

Original variables	Factor1	Factor2
#X disomy_62-76	0.96650	-0.23119
#X disomy_58-80	0.94894	-0.05406
XXY+XX,-Y_62-76	0.95938	-0.23271
#X disomy_66-72	0.69858	0.71287
% of Explained Variance	81.1	15.5

As evident from Table 3, the analysis ended up into a two-component solution cumulatively explaining 96% of the total variance. The #X-disomy distribution highlighted a striking correlation structure, with a major "size" [40], first principal component (Factor1), and a minor "shape" component. The presence of very high and positive loadings (Pearson correlation coefficient between the original variables and components) as for Factor1 corresponds to the fact, that all the categories contribute "along the same direction" to the Factor1 scores and thus, it is an integrated score of the "amount of #X-disomy". The second "shape" component (Factor 2) explains 15% of the total variance and this value mainly stems from the unique mesothelioma pattern (see Fig.6). It is worth noting that principal components are orthogonal to each other by construction and therefore, "size" and "shape" are two independent latent concepts [42]. PCA thus showed an extremely ordered #X-disomy distribution in near-triploid space of all tumour types. The presence of such a strong correlation structure is indicative of the non-random character of #X-disomy distribution in triploidy. As a matter of fact, a maximum likelihood approach to factor extraction [41] highlighted a very high statistical significance (Bartlett-corrected Chi-Square = 87.01, $p < 0.0001$) against the null hypothesis of no common factor.

Commenting space distribution of #X-disomy for different tumour types, it should be noted that 12 of 14 somatic tumour types form a common "shape" cluster with the germ tumour seminoma underlying a common biological phenomenon. The peculiarity of mesothelioma (Nr 12), may be due to its (still unknown) highly plastic progenitors switching between different phenotypes depending on the local microenvironment [43]. Thus, the PCA results showed an extremely ordered X-chromosome disomy distribution in near-triploid space of all tumour types. The presence of such a strong correlation structure is indicative of the non-random character of #X-disomy distribution in triploidy. The emergence of a by far major "size" component points toward a largely invariant pattern of

disomy distribution among tumour types that mainly differ for the amount of #X-disomy keeping the relative frequency of various tumour types largely constant.
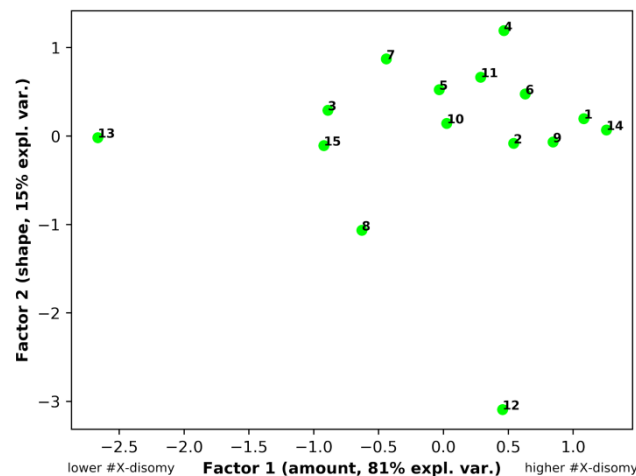
Figure 6. PCA results of the X-chromosome disomy distribution in the triploidy space for 15 malignant tumour types designated as numbered in Table 1.

4. Discussion

In this study, we hypothesised that tumour near-triploidy, which is associated with chemoresistance, may originate primarily from the rearrangement of whole-genome complements. Therefore, we attempted to differentiate it from aneuploidy resulting from chromosomal aberrations. As a method, we chose to analyse the sets of sex chromosomes in their relation to modal chromosome numbers in male tumour karyotypes. Through all analysed material representing 20 types of epithelial and mesenchymal, somatic and germ, malignant and benign male tumour 2928 karyotypes we found that near-triploid karyotypes were characterized by X-chromosome disomy.

The simplest explanation for #X-disomic chromosomes would be of their origin from missegregation of sister chromatids in aberrant mitosis. Random missegregation of individual chromosomes is a well-known mitotic aberration in breakage-fusion-bridge cycles. Tumour aneuploidy concerning the re-arrangements of whole chromosomes and near-triploidy, in particular, has been hitherto explained mostly from the position of the random aberrations in mitotic cycles [2]. However, Pearson correlations carried out in our study established that a kind of aneuploidy represented by #X-disomy (with the dominating XXY karyotypes) showed a very high correlation ($r \sim 0.9$, $p < 0.001$) with triploidy, while PCA resulted in an extremely ordered X chromosome disomy distribution in near-triploid space of all tumour types clearly indicating a departure from randomness.

As well, #X-disomic tumours were clearly repulsing from the near-diploid chromosome range with a strong negative correlation, and thus collectively, from the null hypothesis of the random origin of X chromosome missegregation.

It is also worth noting, in addition, that a dominating set of #X-disomy in male cells was XXY, while the XYY sets, which at random choice would have 50% chance, were practically non-existent in the dataset (only one among 2928 analyzed male tumour karyotypes being near-triploid XYY). This indicates that #X-disomy and near-triploidy of male tumour karyotypes are two facets of the same phenomenon, not stemming from random aberrant chromosome missegregation in a mitotic cycle.

We also found that somatic tumour types and seminoma not only highly correlated together with #X-disomy related to near-triploidy by Pearson coefficient and p-value but also constituted a common "shape" cluster in PCA space. So, the identified novel phenomenon of the persuasive link between #X-disomy and near-triploidy dominated by

the "feminized" XXY karyotype may not be of a mitotic origin. Rather, it may possess meiotic features directed toward the oogenic pathway.

Although the above analysis points towards the concerted missegregation of the chromosome complement of the maternal genome, which would be expected in a process similar to oocyte maturation, we have not yet elaborated the direct data establishing this process. However, the study of Ozery-Flato et al [44] on the same Mitelman database including 15,000 karyotypes of 62 tumour cohorts, among them 18 solid (some the same as we have explored), analysing all aberrations identifiable by cytogenetic techniques revealed the strongest association among mainly whole chromosome gains and losses, with the gains prevailing. This regularity was also confirmed by comparative genome hybridisation analysis. The data of these researchers are supportive for our interpretation of XXY male tumour triploid karyotypes as resulting from the whole genome complements rearrangements of the meiotic (for somatic tumours, pseudo-meiotic) origin.

How may this process proceed? XXYY sets could possibly serve as a starting point. Configuration of sex chromosomes XXYY was found present in each malignant tumour cohort of our tumour sets (except for gastric carcinoma). It is particularly high in seminoma (10.3%). Although designated as triploid XXY,+Y (see the last column in Table 1), it can derive either from spermatocytes I or from G2/mitotic slippage fraction XXYY of any tumour. #X-disomic karyotypes didn't display a statistically significant correlation with tetraploidy. (Fig.2F). In other words, it does not prove but also does not deny that the formation of XXY triploid karyotypes for male tumours starts from the G2/mitotic slippage (XXYY) phase as a meiosis-like process.

Molecularly and particularly in the case of reprogramming, this mito-meiotic trigger is feasible because the G2 mitotic recombination checkpoint is molecularly identical to the recombination checkpoint of the meiotic prophase and evolutionarily derived from it [45]. The reprogramming shift may be favoured by cell senescence and associated DNA damage [46], CIN itself [3], and also by activation of the RAS-RAF/MOS-MEK-MAPK pathway, where the up-regulated RAS is substitutive for MOS in oocyte maturation and is involved in fertilisation [46,47]. Quite in accord, human somatic tumours ectopically express meiotic genes and proteins and also enhance their synthesis after genotoxic challenge [48–54], while primordial male germ cells and their immediate progeny are able to undergo oogenesis in adverse conditions [55].

If so, a process similar to sexual digyny (aberrant fusion of an unreduced maternal diploid gamete with non-disjunct sister chromatids and a paternal haploid gamete) occurring here in a parasexual, pedogamic manner could explain the persuasive formation of XXY triploid karyotypes in a proportion of male tumours. It is schematically presented on Fig.7 as starting from the mitotic G2 recombination checkpoint gliding into a meiotic prophase-like state.

The digyny-like process can use meiotic recombination for effective repair of DNA damage and gene conversion, while the third genome can compensate recessive lethal mutations (and also moderate the genome imbalance created by the inevitably joined CIN). Thus, this variant of the whole genome aberrations in triploid tumours can favour both their perpetuation and chemoresistance. More information on the evolutionary significance of digyny and alternating of its cycle with a mitotic cycle in triploid tumours can be found in the paper of Salmina et al. "When three isn't a crowd: A Digyny concept for treatment-resistant, near-triploid human cancers" [56].

This leads us to question if these revelations have any potentially predictive clinical significance. To this end, it is interesting to compare somatic tumours and seminoma, on one side, and colon adenoma and carcinoma, on the other side. Table 1 shows that seminoma has the highest proportion of XXY karyotypes (47%) and also a fraction of XX,-Y - likely due to the secondary loss of Y-chromosome (together 56%), however possesses a relatively small fraction (5%) of less stable #X-disomic karyotypes (XY,+X)+(X,-Y,+X). On

the contrary, somatic tumours display a higher proportion of more defective but still keeping to #X-disomy karyotypes and they still negatively correlate with near-diploidy. This points towards a primary origin of XXY triploidy and secondary origin of unstable #X-disomic karyotypes derived from it and also highlights the fact that somatic tumours have higher secondary CIN involving loss of single chromosomes (#Y and autosomes) than seminoma. Quite in accord with our suggestion that seminoma is less subjected to secondary CIN alterations, it was found that, except a few driver mutations, seminoma has far less secondary passenger mutations in comparison with somatic cancers [57]. Deeper insights into the predictive potential of our findings need further work.
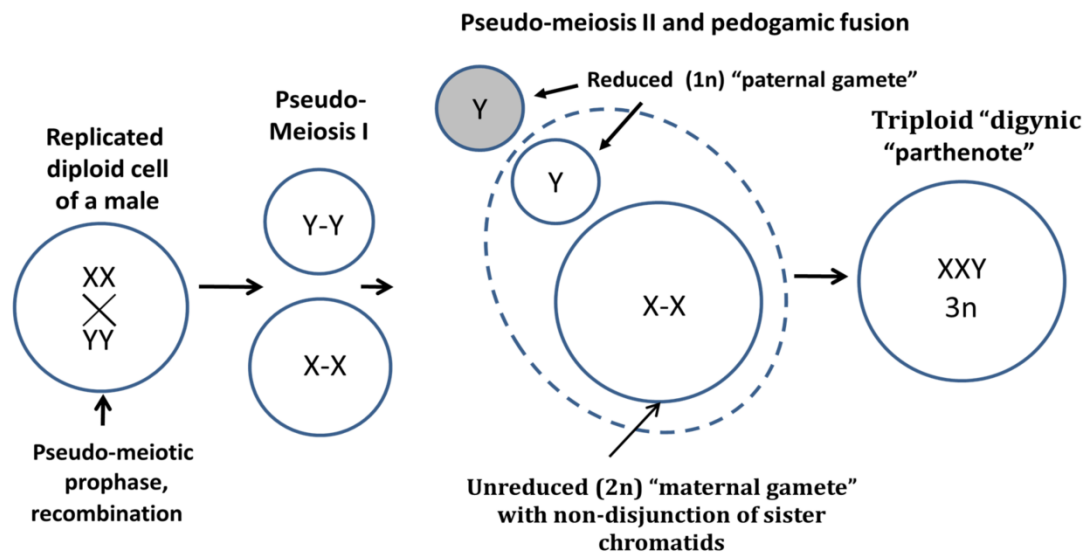
Figure 7. Schematic of the digyny-like formation of XXY triploid karyotypes in somatic male tumours. The reprogrammed male tumour cell triggers the aberrant molecular pathway of the pseudo-meiotic prophase from G2-phase, undergoes recombination between cohesed sisters and possibly, also homologues, pseudo-meiosis I segregating maternal and paternal progenies with cohesed sister chromatids, reduction to haploidy of the "paternal gamete" in the pseudo-meiosis II and its pedogamic fusion with the unreduced diploid "maternal gamete" resulting in triploid "digynic parthenote".

5. Conclusions

The analysis of karyotypes of 15 male malignant tumour types, germ and somatic, from the Mitelman database revealed the very high correlation between X chromosome disomy (predominantly represented by XXY karyotypes) with triploidy, negative correlation with near-diploidy and no correlation with near-tetraploid modal chromosome numbers. In addition, Principal Component Analysis revealed the strongly non-random nature of the #X-disomy and triploidy association and clustering of the germ tumour seminoma with somatic tumours in the PCA space. Collectively, it suggests that the disomy of the X chromosome and triploidy in the typically XXY karyotypes in male tumours represent two facets of the same biological phenomenon - the rearrangement of the whole genome complements. A hypothesis of a sexual digyny-like process (aberrant fusion of two maternal genomes with one paternal) exploiting the elements of meiosis in reprogrammed tumour cells has been proposed. The analysis of partly defective XXY triploid karyotypes still keeping to #X-disomy allows suggesting that chromosome instability may be largely secondary to the whole genome complement rearrangements. The potential for clinical predictions based on the comparison of colon adenoma and carcinoma and of seminoma with somatic malignant tumours is preliminarily discussed.

Author Contributions: conceptualization, Je.E.; methodology, R.K., NMV, P.Z. and A.G.; validation, NMV, A.G. and P.Z.; formal analysis P.Z; investigation, NMV and A.G.; data curation, P.Z. and R.K., writing-original draft preparation, NMV; writing- review and editing, Je.E.; supervision, Je.E.

Funding: This work has been supported by a grant of the European Regional Development Fund (ERDF) project No. 1.1.1.1/18/A/099 and Alfred Raister memorial scholarship to NMV.

Acknowledgments: The cytogenetic advice of Dr Aigars Dzalbs is highly appreciated.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Holland, A.J.; Cleveland, D.W. Losing balance: the origin and impact of aneuploidy in cancer. *EMBO Rep.* **2012**, *13*, 501–514.
2. Pfau, S.J.; Amon, A. Chromosomal instability and aneuploidy in cancer: from yeast to man. *EMBO reports* 2012, *13*, 515–527.
3. Pihan, G.A. Centrosome dysfunction contributes to chromosome instability, chromoanagenesis, and genome reprograming in cancer. *Front. Oncol.* **2013**, *3*, 277.
4. Heng, H.H.; Bremer, S.W.; Stevens, J.B.; Horne, S.D.; Liu, G.; Abdallah, B.Y.; Ye, K.J.; Ye, C.J. Chromosomal instability (CIN): what it is and why it is crucial to cancer evolution. *Cancer Metastasis Rev.* **2013**, *32*, 325–340.
5. Liu, G.; Stevens, J.B.; Horne, S.D.; Abdallah, B.Y.; Ye, K.J.; Bremer, S.W.; Ye, C.J.; Chen, D.J.; Heng, H.H. Genome chaos: survival strategy during crisis. *Cell Cycle* **2014**, *13*, 528–537.
6. Ye, C.J.; Regan, S.; Liu, G.; Alemara, S.; Heng, H.H. Understanding aneuploidy in cancer through the lens of system inheritance, fuzzy inheritance and emergence of new genome systems. *Mol. Cytogenet.* **2018**, *11*, 31.
7. Weaver, B.A.A.; Silk, A.D.; Montagna, C.; Verdier-Pinard, P.; Cleveland, D.W. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* **2007**, *11*, 25–36.
8. Sheltzer, J.M.; Ko, J.H.; Replogle, J.M.; Habibe Burgos, N.C.; Chung, E.S.; Meehl, C.M.; Sayles, N.M.; Passerini, V.; Storchova, Z.; Amon, A. Single-chromosome Gains Commonly Function as Tumor Suppressors. *Cancer Cell* **2017**, *31*, 240–255.
9. Raikov, I.B. *The Protozoan Nucleus*; Cell Biology Monographs; Springer-Verlag Wien, 1982; Vol. 9; ISBN 9783709141380.
10. Demin, S.Y.; Berdieva, M.A.; Goodkov, A.V. Cyclic polyploidy in obligate agamic amoebas Cell Tiss. Biol. **2019** 13: 242. http://sci-hub.tw/10.1134/S1990519X1903002711.
11. Salmina, K.; Jankevics, E.; Huna, A.; Perminov, D.; Radovica, I.; Klymenko, T.; Ivanov, A.; Jascenko, E.; Scherthan, H.; Cragg, M.; et al. Up-regulation of the embryonic self-renewal network through reversible polyploidy in irradiated p53-mutant tumour cells. *Exp. Cell Res.* **2010**, *316*, 2099–2112.
12. Vincent, M.D. Cancer: beyond speciation. *Adv. Cancer Res.* **2011**, *112*, 283–350.
13. Trigos, A.S.; Pearson, R.B.; Papenfuss, A.T.; Goode, D.L. Altered interactions between unicellular and multicellular genes drive hallmarks of transformation in a diverse range of solid tumors. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 6406–6411.
14. Vinogradov, A.E.; Anatskaya, O.V. Evolutionary framework of the human interactome: Unicellular and multicellular giant clusters. *Biosystems.* **2019**, *181*, 82–87.
15. Vazquez-Martin, A.; Anatskaya, O.V.; Giuliani, A.; Erenpreisa, J.; Huang, S.; Salmina, K.; Inashkina, I.; Huna, A.; Nikolsky, N.N.; Vinogradov, A.E. Somatic polyploidy is associated with the upregulation of c-MYC interacting genes and EMT-like signature. *Oncotarget* **2016**, *7*, 75235–75260.
16. Erenpreisa, J.; Giuliani, A.; Vinogradov, A.E.; Anatskaya, O.V.; Vazquez-Martin, A.; Salmina, K.; Cragg, M.S. Stress-induced polyploidy shifts somatic cells towards a pro-tumourogenic unicellular gene transcription network. *Cancer Hypotheses* **2018**, *1*, 1–20.

17. Anatskaya, O.V.; Erenpreisa, J. Polyploidy reprograms regulatory pathways towards unicellular mode: the role in stress response, drug resistance, growth and cancer. In Proceedings of the Biopolymers & Cell 2019, 35 (3); 2019; pp. 188–189.

18. Kondrashov, A.S. Evolutionary Genetics of Life Cycles. *Annual Review of Ecology and Systematics* 1997, *28*, 391–435.

19. Kondrashov, A.S. The asexual ploidy cycle and the origin of sex. *Nature* **1994**, *370*, 213–216.

20. Rajaraman, R.; Rajaraman, M.M.; Rajaraman, S.R.; Guernsey, D.L. Neosis--a paradigm of self-renewal in cancer. *Cell Biol. Int.* **2005**, *29*, 1084–1097.

21. Erenpreisa, J.; Cragg, M.S. Cancer: a matter of life cycle? *Cell Biol. Int.* **2007**, *31*, 1507–1510.

22. Erenpreisa, J.; Cragg, M.S. MOS, aneuploidy and the ploidy cycle of cancer cells. *Oncogene* **2010**, *29*, 5447–5451.

23. Erenpreisa, J.; Kalejs, M.; Cragg, M.S. Mitotic catastrophe and endomitosis in tumour cells: an evolutionary key to a molecular solution. *Cell Biol. Int.* **2005**, *29*, 1012–1018.

24. Gerashchenko, B.I.; Salmina, K.; Eglitis, J.; Huna, A.; Grjunberga, V.; Erenpreisa, J. Disentangling the aneuploidy and senescence paradoxes: a study of triploid breast cancers non-responsive to neoadjuvant therapy. *Histochem. Cell Biol.* **2016**, *145*, 497–508.

25. Schulze, S.; Petersen, I. Gender and ploidy in cancer survival. *Cell. Oncol.* **2011**, *34*, 199–208.

26. Di Oto, E.; Monti, V.; Cucchi, M.C.; Masetti, R.; Varga, Z.; Foschini, M.P. X chromosome gain in male breast cancer. *Hum. Pathol.* **2015**, *46*, 1908–1912.

27. Yamamoto, K.; Nagata, K.; Kida, A.; Hamaguchi, H. Acquired gain of an X chromosome as the sole abnormality in the blast crisis of chronic neutrophilic leukemia. *Cancer Genet. Cytogenet.* **2002**, *134*, 84–87.

28. Okada, Y.; Nishikawa, R.; Matsutani, M.; Louis, D.N. Hypomethylated X chromosome gain and rare isochromosome 12p in diverse intracranial germ cell tumors. *J. Neuropathol. Exp. Neurol.* **2002**, *61*, 531–538.

29. Hunter, S.; Gramlich, T.; Abbott, K.; Varma, V. Y chromosome loss in esophageal carcinoma: An in situ hybridization study. *Genes Chromosomes Cancer* **1993**, *8*, 172–177.

30. Park, S.-J.; Jeong, S.-Y.; Kim, H.J. Y chromosome loss and other genomic alterations in hepatocellular carcinoma cell lines analyzed by CGH and CGH array. *Cancer Genet. Cytogenet.* **2006**, *166*, 56–64.

31. Bianchi, N.O. Y chromosome structural and functional changes in human malignant diseases. *Mutat. Res.* **2009**, *682*, 21–27.

32. Duijf, P.H.G.; Schultz, N.; Benezra, R. Cancer cells preferentially lose small chromosomes. *Int. J. Cancer* **2013**, *132*, 2316–2326.

33. Castedo, S.M.; de Jong, B.; Oosterhuis, J.W.; Seruca, R.; te Meerman, G.J.; Dam, A.; Schraffordt Koops, H. Cytogenetic analysis of ten human seminomas. *Cancer Res.* **1989**, *49*, 439–443.

34. Mitelman, F.; Johansson, B.; Mertens, F. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer Available online: http://cgap.nci.nih.gov/Chromosomes/Mitelman (accessed on 2019).

35. Onaitis, M.; Hanna, J. Cell of origin of lung cancer. *J. Carcinog.* **2013**, *12*, 6.

36. Oliphant, T.E. *A guide to NumPy*; USA: Trelgol Publishing, 2006;.

37. McKinney, W. Data Structures for Statistical Computing in Python. In Proceedings of the Proceedings of the 9th Python in Science Conference; van der Walt, S. 'efan, Millman, J., Eds.; 2010; pp. 51–56.

38. Jones, E.; Oliphant, T.; Peterson, P. SciPy: Open Source Scientific Tools for Python Available online: http://www.scipy.org/ (accessed on 2019).

39. Shaffer, L.G.; McGowan-Jordan, J.; Schmid, M.; International Standing Committee on Human Cytogenetic Nomenclature *ISCN 2013: An International System for Human Cytogenetic Nomenclature (2013)*; Shaffer, L.G., McGowan-Jordan, J., Schmid, M., Eds.; Karger Medical and Scientific Publishers, 2013; ISBN 9783318022537.

40. Jolicoeur, P.; Mosimann, J.E. Size and shape variation in the painted turtle. A principal component analysis. *Growth* **1960**, *24*, 339–354.

41. Bickel, P.J.; Doksum, K.A. *Mathematical statistics: basic ideas and selected topics*; San Francisco: Holden-Day, 1977;.

42. Giuliani, A. The application of principal component analysis to drug discovery and biomedical data. *Drug Discov. Today* **2017**, *22*, 1069–1076.

43. Herrick, S.E.; Mutsaers, S.E. Mesothelial progenitor cells and their potential in tissue engineering. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 621–642.

44. Ozery-Flato, M.; Linhart, C.; Trakhtenbrot, L.; Izraeli, S.; Shamir, R. Large-scale analysis of chromosomal aberrations in cancer karyotypes reveals two distinct paths to aneuploidy. *Genome Biol.* **2011**, *12*, R61.

45. Nasmyth, K. Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* **2001**, *35*, 673–745.

46. Erenpreisa, J.; Cragg, M.S. Three steps to the immortality of cancer cells: senescence, polyploidy and self-renewal. *Cancer Cell Int.* **2013**, *13*, 92.

47. Erenpreiss, J. *Current concepts of malignant growth. Part A: From a normal cell to cancer*; Zvaigzne: Riga, 1993;.

48. Kalejs, M.; Ivanov, A.; Plakhins, G.; Cragg, M.S.; Emzinsh, D.; Illidge, T.M.; Erenpreisa, J. Upregulation of meiosis-specific genes in lymphoma cell lines following genotoxic insult and induction of mitotic catastrophe. *BMC Cancer* **2006**, *6*, 6.

49. Ianzini, F.; Kosmacek, E.A.; Nelson, E.S.; Napoli, E.; Erenpreisa, J.; Kalejs, M.; Mackey, M.A. Activation of meiosis-specific genes is associated with depolyploidization of human tumor cells following radiation-induced mitotic catastrophe. *Cancer Res.* **2009**, *69*, 2296–2304.

50. Vitale, I.; Senovilla, L.; Jemaà, M.; Michaud, M.; Galluzzi, L.; Kepp, O.; Nanty, L.; Criollo, A.; Rello-Varona, S.; Manic, G.; et al. Multipolar mitosis of tetraploid cells: inhibition by p53 and dependency on Mos. *The EMBO Journal* 2010, *29*, 1272–1284.

51. Gorgoulis, V.G.; Zacharatos, P.; Mariatos, G.; Liloglou, T.; Kokotas, S.; Kastrinakis, N.; Kotsinas, A.; Athanasiou, A.; Foukas, P.; Zoumpourlis, V.; et al. Deregulated expression of c-mos in non-small cell lung carcinomas: relationship with p53 status, genomic instability, and tumor kinetics. *Cancer Res.* **2001**, *61*, 538–549.

52. Erenpreisa, J.; Cragg, M.S.; Salmina, K.; Hausmann, M.; Scherthan, H. The role of meiotic cohesin REC8 in chromosome segregation in γ irradiation-induced endopolyploid tumour cells. *Experimental Cell Research* 2009, *315*, 2593–2603.

53. Chung, J.-Y.; Kitano, H.; Takikita, M.; Cho, H.; Noh, K.H.; Kim, T.W.; Ylaya, K.; Hanaoka, J.; Fukuoka, J.; Hewitt, S.M. Synaptonemal complex protein 3 as a novel prognostic marker in early stage non-small cell lung cancer. *Hum. Pathol.* **2013**, *44*, 472–479.

54. Lindsey, S.F.; Byrnes, D.M.; Eller, M.S.; Rosa, A.M.; Dabas, N.; Escandon, J.; Grichnik, J.M. Potential role of meiosis proteins in melanoma chromosomal instability. *J. Skin Cancer* **2013**, *2013*, 190109.

55. Wang, L.; Cao, J.; Ji, P.; Zhang, D.; Ma, L.; Dym, M.; Yu, Z.; Feng, L. Oocyte-like cells induced from mouse spermatogonial stem cells. *Cell Biosci.* **2012**, *2*, 27.

56. Salmina, K.; Gerashchenko, B.I.; Hausmann, M.; Vainshelbaum, N.M.; Zayakin, P.; Erenpreiss, J.; Freivalds, T.; Cragg, M.S.; Erenpreisa, J. When three isn't a crowd: A Digyny concept for treatment-resistant, near-triploid human cancers. Accepted *Genes,* June 19, **2019**.

57. Cutcutache, I.; Suzuki, Y.; Tan, I.B.; Ramgopal, S.; Zhang, S.; Ramnarayanan, K.; Gan, A.; Lee, H.H.; Tay, S.T.; Ooi, A.; et al. Exome-wide Sequencing Shows Low Mutation Rates and Identifies Novel Mutated Genes in Seminomas. *Eur. Urol.* **2015**, *68*, 77–83.