

Mutagenic Chemotherapy Exposure Precluding Gamete Preservation in Cancer Patients

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Abstract

Concerns for cancer therapy-related infertility rest largely upon the effect on hormonal and gonadal function, resulting in decreased gonadal reserve or premature failure. Recommendations for germ cell preservation are focused on these concerns but do not account for prior chemotherapy exposure that may potentially result in genetic mutations, possibly leading to birth defects. This investigational analysis is offered as a means to discern those agents and exposures which should be identified and preclude gamete preservation. An original analysis examining the documented pharmacokinetics and mutagenic toxicities of conventional chemotherapy was conducted. These results were then compared to administration rates of such agents at our institution. A substantial 54% of investigated agents (and metabolites) were found to be of mutagenic capability. The distribution pattern revealed mutagenic exposures within greater than one-third of the treated child-bearing population, an age bracket representing 27% of the total patient population. With greater than half of conventional chemotherapies having the capability to alter the DNA of oocytes and sperm, determination of prior exposures to mutagenic chemotherapeutics should be undertaken. If exposed, preclusion of gamete preservation in such circumstances may be warranted secondary to possible increased risk of genetic mutation potentially leading to birth defects, both physical and mental. Identifying such exposures in reproductive age patients fundamentally changes the current approach to fertility preservation.

Keywords: Chemotherapy, Fertility, Mutagen, Mutagenic, Oncofertility

Introduction

A misconception regarding cancer survivorship is that it is an affliction of the older population, despite the fact there are still those of reproductive age facing the same diagnosis. A 2014 estimate projected 14.5 million cancer survivors in the United States, with approximately 7% under the age of 40 [1]. While males have a greater *probability* of successful fertility at ages greater than 40, females retain the *possibility* of conceiving, especially with advancements in fertility technology and treatments. Incorporating cancer survivors aged 40-49, the population at risk for treatment related infertility is approximately 12%, or an estimated 1.7 million. Over time, as the population of cancer survivors increases, so will the number of childbearing-age patients [1].

Early identification of those at risk, as emphasized by the American Society for Clinical Oncology (ASCO), facilitates a timely evaluation and discussion regarding the patients' concerns as well as medical appropriateness for preservation [2]. Although the effects of both chemo- and radiotherapies on ovarian and testicular reserve are well documented, the mutagenic and/or clastogenic effects of chemotherapy *in addition* to radiotherapy should also be considered.

Materials and Methods

Mutagenic/clastogenic changes comparing radiotherapy to chemotherapy

Radiotherapy: Focused-beam radiation therapy to the abdomen and pelvis, regardless of reproductive organ targeting, can result in reduction or destruction of gonadal function. It may also result in gametal chromosomal damage with the possibility of indefinite persistence [3-9]. While some organs may be more aptly protected externally, lead shielding does not prevent internal radiation scatter, which could have a detrimental effect of gonadotoxicity. Increased distance from focal-beam localization may result in decreased peripheral photon dosing, which may have less of a deleterious gonadal effect [9-13]. Due to adverse effects of photon exposure, prior direct or relative

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gonadal exposure to dedicated radiotherapy within the confines of the pelvis is contraindicated for fertility preservation.

Chemotherapy: Chemotherapy can result in potential premature gonadal failure and chromosomal aberrations [3,5,6,8,12,14]. There are individual chemotherapies, as well as chemotherapeutic classes, which have a more pronounced effect in this regard. Chemotherapies considered mutagenic, (an agent which induces genetic mutation) or clastogenic (causing disruption or breakages of chromosomes) based on their representative determination assays, present a confounding factor determining appropriateness of gamete preservation with prior chemotherapeutic exposure [15,16]. An original analysis of 140 discrete medications within pharmacology and toxicology reports was performed. Parameters used were carcinogenicity, mutagenicity and impairment of fertility with delineated results summarized in Figure 1. These results were then compared to retrospective administration rates of such agents at our institution.

Results

Variability with the inferred definition of the term genotoxic, pertaining to radiation or chemical agents known to damage DNA, was observed. Many utilize the terms mutagenic and clastogenic as previously defined, with genotoxic referring to overall carcinogenicity (the ability or tendency to produce cancer) [15]. Those which were specific to include negative assay results for mutagenicity and clastogenicity but were found to be genotoxic were interpreted as implying carcinogenesis.

Dissection of each chemotherapeutic class revealed patterns specifically involving alkylating agents, antimetabolites, antibiotic oncologics and mitosis inhibitors, with large proportions of their classes documented as a known mutagen or clastogen (Figure 1). Researching each class individually, it was difficult to identify claims of specific genetic defect outside of the animal model, with identification methods limited mainly to observation of congenital defects from natural conception achieved after cancer therapy was administered. With assisted reproductive technology

(ART), the limitations of natural conception post-cancer therapy can potentially be overcome, circumventing the body's inherent restriction of latent mutated germ cells and gametes. The above named chemotherapies can be defined as those which have direct effects on DNA and/or RNA, with discrete mutagenic or clastogenic patterns.

These agents have largely been investigated for mutagenicity and clastogenicity via the animal model. Despite substantial evidence, results from the animal models were often not extrapolated to human germ cells due to limitations in technology, lack of understanding of the susceptibility of male and female gametogenesis to mutation and need for strategized research. Additionally, mutagenicity may not be limited entirely to the first generation inheritant; de novo epigenetic changes can have a trans-generational effect [17,18].

Despite limitations in identifying and documenting mutagenic and clastogenic effects on human germ cells, alternative approaches can be taken to determine if a particular medication is cause for concern. In examining clinical pharmacologies and pharmacokinetics of each chemotherapeutic deemed mutagenic or clastogenic, another compelling pattern emerged. Almost every agent tested was found to have a percentage of agent or major metabolite unaccounted for after pharmacokinetic elimination studies for radiolabeled total mean recovery were conducted, with varying degrees of identification of active metabolites. Approximately one half of the agents in the identified classes are bound to plasma proteins, some of which are irreversible and most at a significantly elevated rate (Table 1). Medications which undergo significant plasma protein binding can considerably lengthen the chemical elimination process from the body, prolonging exposure of germ cells and gametes to mutagenic substances and some potential metabolites of the original agent yet to be identified or quantified. Some of these agents and/or metabolites are also lipophilic, allowing potential absorption into bodily fat stores, delayed release, and maintenance of persistent subtherapeutic levels of chemical exposure [19].

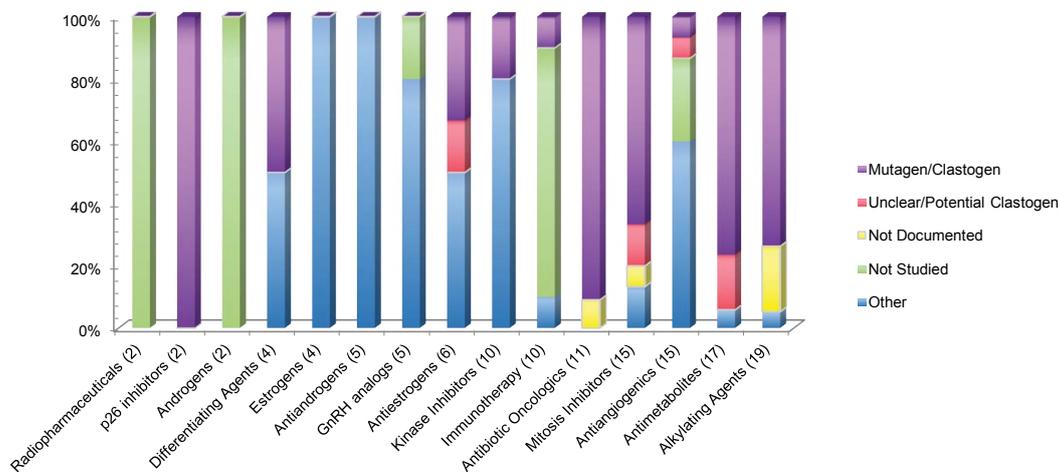


Figure 1: Comparison of Mutagenic/Clastogenic Chemotherapeutics vs. Total Agents in Class

Graphic representation of the tabular findings of chemotherapeutic classes and number of agents found to be mutagenic within. Four classes of chemotherapies are noted to have a striking representation of noted mutation when compared with the total number of agents residing in that class. Other – includes no mutagenic activity found, not reliable, inconclusive and genotoxic findings

Chemotherapeutic Agent/Class	Total Agents	Plasma Protein Bound ^a	Documented Lipophilic Ability ^a	Total Mean Recovery ^a	Renal Excretion ^a	Fecal Excretion ^a	Other Excretion ^a	Time ^a	Percent Unaccounted ^a
Alkylating Agents	19	6-96% ^b	7	10-90% ^c	10-86% ^c	0.8-25%	Respiratory, Hydrolysis	6hrs-7days	10-80% ^{d,e}
Antimetabolites	17	20-81%	2	32-98%	18-92.9%	<1-2.6%	Respiratory, Biliary	4hrs-7days	1-68%
Antibiotic Oncologics	11	70-97%	2	60-99%	4-99% ^c	27-50%	Biliary	1-9 days	1-64%
Mitosis Inhibitors	15 ^d	30-98%	1	18-100%	3.7-44%	<10-82%	Biliary	2-14 days	0-82%
Other ^f	14	71-99.8%	—	71-96%	4-66.4%	19.8-85%	—	3-14 days	4-29%

^a of agents reported; not all agents studied/clearly documented

^b some agents bound irreversibly

^c majority <= 50%

^d majority >= 50%

^e per pharmacokinetics report: one agent/metabolite "present in tissues at least 180 days"

^f arsenic trioxide, bortezomib, carfilzomib, dasatinib, exemestane, gemtuzumab ozogamicin, imatinib, megestrol, porifimer, regorafenib, sorafenib, toremifene, tretinoin, vorinostat

Table 1: Radiolabeled Elimination Patterns of Chemotherapeutics found to be Mutagenic/Clastogenic

Potential Mutagenic Exposure in Reproductive Age Patients

Of the 140 previously noted agents in Figure 1, 76 were found to have mutagenic impact or potential through class effect. This represents a substantial 54% of commonly available or utilized therapies with gene altering ability. Recognizing the significant proportion of mutagens this indicated, it compelled further investigation into the pattern of recipient exposure.

An analysis of the patient population at our institution was performed to quantify the frequency of employment of mutagenic/clastogenic chemotherapies, as well as their distribution across varying age groups. Administration of all identified mutagenic agents in 2014 was tabulated by patient to avoid redundancy through multiple treatment encounters. Results were further delineated to reflect concentration of distribution according to average child-bearing (≤ 49 yo) and older (≥ 50 yo) populations.

Taking the patient population as a whole, 27% falls within the reproductive age bracket. This population evaluation has demonstrated an astonishing 34% of our child-bearing age demographic have been exposed to identified mutagenic/clastogenic pharmaceuticals (Figure 2). Reflecting on the gene-altering ability these medications could have, the inherent risk of

genetic and chromosomal aberrations manifesting in discernable birth defects could be logically extrapolated to be higher than that of the average population.

Discussion

Inferences can be drawn by combining the evidence from each medication's documented mutagenicity or clastogenicity, unaccounted for chemotherapeutics and/or potential metabolites, and propensity for delayed elimination due to high rates of plasma protein binding and lipid solubility.

First, it is plausible for germ cells and gametes to have protracted exposure to mutagenic or clastogenic agents, possibly indefinitely, based on rates of plasma protein liberation, fat stores and persistent re-exposure for planned treatment.

Secondly, it is possible for continued exposure to mutagens from metabolite formation, both known and unknown. As those which have yet to be identified have not been quantified, their mutagenicity, plasma protein binding capability, lipophilicity and elimination potential may be equally detrimental.

Thirdly, four classes of chemotherapeutics evaluated can be distinguished based on their significantly elevated rates of identified mutagenicity or clastogenicity. Combination of

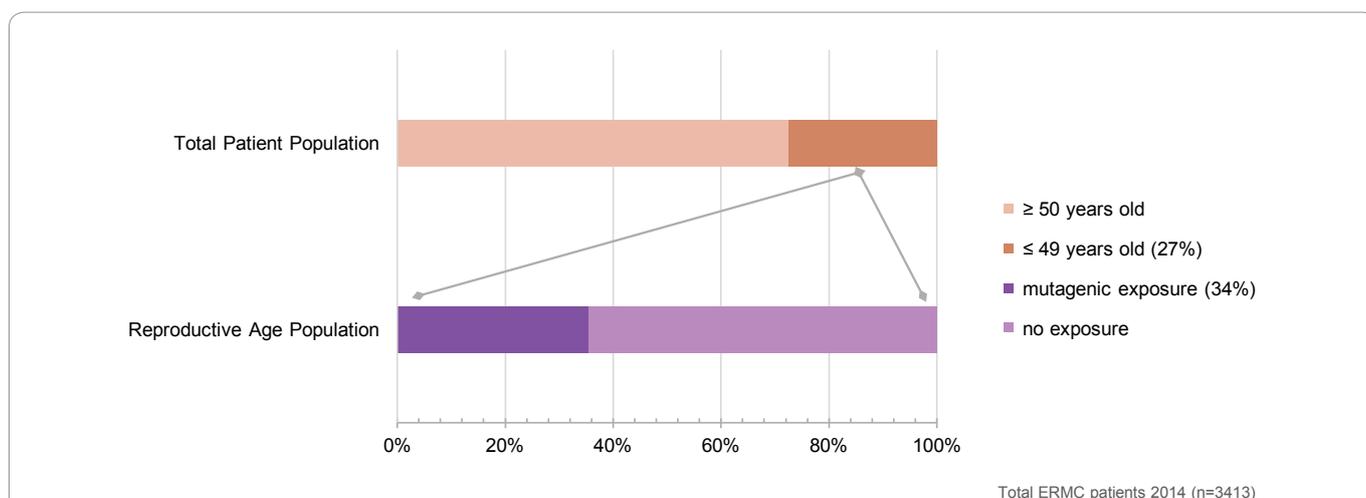


Figure 2: Mutagenic/Clastogenic Chemotherapy Exposure within Reproductive Age Population

Graphic representation of mutagenic/clastogenic chemotherapy exposures within the child-bearing population (≤ 49 years old) at ERM in 2014. When compared to the total population, the reproductive bracket represents 27% of the total population, with 34% of this grouping having mutagenic chemotherapy exposure.

their known cytotoxic effects and pattern of observations from individual pharmacology and toxicology reviews with the aforementioned conclusions argue for the feasibility of implementation of class effect, regardless of whether an agent was prior studied.

Lastly, all those agents with direct effect on DNA and/or RNA, even outside the four classes listed seem to have a consistent, alarming propensity for inducing genetic mutations and chromosomal aberrations. Prior exposure to any of these identified agents should preclude any attempt at fertility preservation.

Identifying mutagenic exposure

Although the risk for diminished gonadal reserve and premature gonadal failure is ever-present with cancer directed therapy, certain forms of treatment can go beyond such risk to include potential genetic mutations. Chromosomal changes and recommendations for gamete preservation prior to treatment with ionizing radiation have been well documented, as previously noted. Taking into consideration that a moderate number of chemotherapies are shown to induce a similar chromosomal effect, it is plausible that these chemotherapeutics can have enduring effects on germ cells and gametes that elevate risk for genetic defect. Consequently, exposure to probable gene-altering chemotherapeutic agents should be taken into consideration when determining appropriateness for gamete preservation.

In accordance with that consideration, if a patient has had prior exposure to ionizing radiation in the pelvic region or chemotherapeutics with mutagenic properties, it is recognized to be inappropriate to recommend preservation of their own gametes based on these findings. Genetic mutations can result in a variety of outcomes, including visual defect (dependent on the type of mutation) as well as those that are not readily visible, such as mental illness [20], which becomes increasingly evident over time. Failure to recognize such exposures prior to gamete retrieval could have potentially disastrous consequences.

Despite these recommendations, patients who are not appropriate for their own gamete preservation are still eligible for discussion regarding donor cells, gestational carriers and adoption with regards to their specific clinical situation and individual concerns.

Conclusion

Early identification and crucial conversations are key to providing the widest array of fertility preservation options while coordinating oncologic therapy. Care should be taken to account for prior chemo and radiotherapeutic exposures when determining appropriateness for gamete cryopreservation. In addition to pelvic irradiation, prior exposures to chemotherapy agents/classes with clastogenic and mutagenic potential should preclude attempts at oocyte or spermatic retrieval, as well as preclude later use of said genetic material due to their noted gene-altering ability. Furthermore, discussions regarding the potentially devastating impact these agents may impose should be conveyed during preservation counseling, with the ultimate

ideal of gamete harvesting occurring prior to the initiation of any cancer-directed therapy.

Acknowledgements

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Conflicts of Interest

The author has declared no conflicts of interest.

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