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REVIEW

Glycoconjugation: An approach to cancer therapeutics

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Abstract

Cancer constitutes the second leading cause of death globally and is considered to have been responsible for an estimated 9.6 million fatalities in 2018. Although treatments against gastrointestinal tumors have recently advanced, those interventions can only be applied to a minority of patients at the time of diagnosis. Therefore, new therapeutic options are necessary for advanced stages of the disease. Glycosylation of antitumor agents, has been found to improve pharmacokinetic parameters, reduce side effects, and expand drug half-life in comparison with the parent compounds. In addition, glycosylation of therapeutic agents has been proven to be an effective strategy for their targeting tumor tissue, thereby reducing the doses of the glycodrugs administered to patients. This review focusses on the effect of the targeting properties of glycosylated antitumor agents on gastrointestinal tumors.

Key words: Glycosylation; Gastrointestinal cancers; Antitumoral agents; Therapeutic strategies; Drug targeting

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Core tip: In nature, glycosylation has proven an effective strategy for expanding the biologic information of biomolecules by adding a new level of structural diversity. The high specificity of the interaction with carbohydrates and the overexpression of carbohydrate receptors in tumoral cells that can be specifically targeted by glycodrugs enable a selective administration of those agents to the tumor tissues. Accordingly, the glycosylation of antitumor agents has been found to improve pharmacokinetic parameters, reduce side effects, expand drug half-life, and reduce the dosage of the consequent glycoderivatives.

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INTRODUCTION

Cancer is the second leading cause of death worldwide, having been responsible for an estimated of 9.6 million deaths in 2018[1,2]. Moreover, 21.6% of all tumors worldwide are gastrointestinal cancers at more than 26000 cases per year[3]. Despite considerable research efforts in recent years, the impact of conventional strategies - including surgery, radiotherapy, and chemotherapy - on the prognosis of tumors has been only moderate[4,5]. The anticancer drugs used in chemotherapy usually target cells that are proliferating. These chemodrugs can be grouped according to their main role: antitumor antibiotics, alkylating agents, topoisomerase inhibitors, DNA-complexing agents, mitotic inhibitors, hormones, and immunotherapeutic agents. The activity of those drugs is essential for predicting side effects; moreover, what is remarkable is that most of the available anticancer drugs have the disadvantage of lacking systems for delivery to the target organ or tissue. Consequently, the majority of the administered drug remains circulating in the bloodstream, thus increasing the side effects on noncancerous cells[6].

The selective targeting of therapeutic agents has several advantages, such as increasing the concentration of the drugs in the tumor and reducing the concentration in other tissues[7]. Carbohydrates *per se*, exhibit a high solubility in water, a low toxicity, and a high biocompatibility; thus constituting an attractive system for facilitating drug-delivery. Glycosylated compounds can be targeted to a broad range of cellular receptors because of the specificity of interaction with cell-surface carbohydrates. Accordingly, a number of glycoconjugated antitumoral agents have been reported to selectively deliver the parent drugs to the desired sites[8,9]. In 1971, Rogers *et al*[10] demonstrated for the first time the usefulness of targeting proteins that bear carbohydrates as ligands. To our knowledge, no therapeutic targeting system is currently on the market, though many candidates aimed at that end nevertheless exist. This review will therefore focus primarily on the synthesis of the most relevant glycosylated therapeutic agents and the effect of those derivatives on gastrointestinal tumors.

GLYCOTARGETING SYSTEMS MEDIATED BY RECEPTORS AND TRANSPORTERS

The most widely studied drug-transporting and receptor-targeting glycoligands are the glucose-transporters (GLUTs) and the lectin receptors as described below.

GLUT

The energy produced from glucose metabolism is essential for sustaining mammalian-cell life. The end products of that metabolic pathway are lactate and, upon full oxidation in the mitochondria, CO_2[11].

In tumors and other proliferating cells, the rate of glucose uptake increases considerably and lactate is produced, regardless of the availability of oxygen and functional mitochondria. The tendency of tumoral tissues to anaerobically metabolize large amounts of glucose, in comparison with noncancerous tissue, is known as the Warburg Effect[12,13].

The GLUT is a transport protein of the facilitator family involved in glucose translocation across the cell membrane. Although, GLUT transporters are expressed in almost every cell type[14], tumor cells express a large number of glucose transporters that are related to poor prognosis[15]. At present, 14 different GLUTs-1 to 14 - have been described[16]. GLUT-1, in particular, is known to be overexpressed in tumor cells, including those of liver, pancreas, and stomach[17]. These transporters can specifically recognize and transport different sugars, such as glucose, mannose, galactose, 2-deoxyglucose and glucosamine analogs[18].

Therefore, as a result of the Warburg effect, designing and developing glycosyl-based targeted drugs is a subject of high/considerable/widespread interest[19,20].

Lectin receptors

Lectins are defined as proteins, usually linked to carbohydrates[21-23], that are present in plants and animals and involved in many biologic processes - including cell growth, differentiation, signalling, adhesion and migration, and apoptosis[24-26]. Lectins can act as receptors, either for binding oligosaccharides to cell membranes or free-floating glycans involving monosaccharides in order to mediate signal transduction and/or drug transport[27].

Asialoglycoprotein receptors (ASGPRs), including asialoglycoprotein receptors 1 and 2-ASGPR1 and ASGPR2 - are expressed on the surface of hepatocytes and stomach and gallbladder epithelia[28] and preferentially interact with the sugars D-galactose and L-rhamnose. The molecular mechanism consists in the internalization of the receptor-ligand complex through a clathrin-mediated endocytosis. Once inside the cell, the ligands are released, enabling the recycling of ASGPR receptors back into the plasma membrane. The quick cycling of internalized receptors is the key process that maintains their concentration on the cell surface. Owing to the high specificity in the binding of galactose and rhamnose to ASGPR receptors, these interactions result a promising approach to drug targeting[29].

Among the lectin-based receptors, the rhamnose-binding lectin receptor ligates specifically to rhamnose and is highly expressed on various tumor-cell types, like the cell-culture lines KB (from a human squamous-cell carcinoma), PC3 (from a human prostatic small-cell carcinoma), HT-29 (from a human colon adenocarcinoma) and MCF-7 (a breast adenocarcinoma)[30]. Although the molecular mechanism involved in the transmission of messages by rhamnose-binding lectin receptor has not been yet studied in humans, the strategy for using rhamnosylated anticancer molecules as novel candidates for pharmacological applications has recently been explored[29].

ANTITUMORAL DRUG GLYCOCONJUGATES

Below we present several antitumoral agents as informative examples of such modifications - namely, ifosfamide, chlorambucil, and paclitaxel. The drugs were linked to various carbohydrate moieties and the antitumoral effects evaluated on gastrointestinal tumors. The cytotoxic activities of the synthesized glycoconjugates were then compared to those of the corresponding parent nonglycosylated molecules (Table 1).

Ifosfamide

Ifosfamide - whose cytotoxic metabolite of in plasma is ifosforamide - is an alkylating agent has been bound to β-D-glucose to form glufosfamide[31,32]. This glycoconjugate was the first molecule bearing a sugar to be explicitly designed and evaluated as a cancer-targeting cytotoxic compound. Within tumor cells, glufosfamide is metabolized by glucosidases to form ifosforamide. This cytotoxic metabolite, in turn, forms DNA crosslinks, therefore inhibiting DNA replication and cell growth[33]. Moreover, treatment with GLUT-1 inhibitors reduced the anticancer efficiency of glufosfamide, suggesting that the drug conjugate was internalized into cells via the GLUT-1 transporter. Finally, glufosfamide was less myelotoxic and presented a higher antitumour activity both *in vitro* and *in vivo* than the parent aglycone[32].

In 1997, the first human clinical trial to test glufosfamide was carried out in Europe, and the results obtained with 20 pancreatic-cancer patients were reported[34]. Two cases evidenced a good response to the treatment, 10 resulted in stable disease, while 8 patients failed to respond. More significantly, one pancreatic-cancer patient in a different trial experienced a complete remission for over 4 years. Since pancreatic-cancer biopsy samples had been found to overexpress GLUT-1; in 2010, Chiorean *et al*[35] performed a phase-II study of glufosfamide plus the nucleoside analog gemcitabine, the standard chemotherapeutic treatment in pancreatic cancer. Glufosfamide and gemcitabine in combination yielded a modest response in two trials on pancreatic-adenocarcinoma patients. In conclusion, glufosfamide appeared to constitute an effective cytotoxic agent exhibiting high antitumor selectivity that was due to an active interaction with the transporter GLUT-1.

Chlorambucil

Chlorambucil is an antineoplasic drug within the class of alkylating agents that is used to treat various forms of cancer[36]. The reactive radical ethylenimonium forms after alkylation that interferes in DNA, RNA, and protein synthesis. The first report of chlorambucil synthesis was over five decades ago[37]. Goff *et al*[38] (2010) synthesized and evaluated a 63-member library of chlorambucil-based neoglycosides in ten different human-carcinoma cell-culture systems, including lung, colorectal, liver,

Table 1 List of the glycoconjugates and its effects

Agent	Activity	Sugar moiety	Efficacy glycoconjugates compared to aglicone
Ifosfamide	Alkalating agent	Glucose	*In vitro* (less toxic) and *in vivo* (reduced tumor size). Clinical trials ongoing.
Chlorambucil	Alkalating and DNA-complexing agent	D-threose	HT29 and HCT15 (showed 8-12 fold, and 15-fold, respectively, improved activities targeting cancers cell lines over the parent drug).
Geldanamycin	HSP90 inhibitor	Glucose	Glucose-GA showed anticancer activity with IC50 of 70.2-380.9 nM in SW620, HT29, MCF-7 and K562 cancer cells by-glucosidase activation inside of the tumor cells.
Geldanamycin	HSP90 inhibitor	Galactose	SW620, HT29, MCF-7 and K562 (anticancer activity of galactose-GE conjugate increased by 3- to 40-fold when incubated with galactosidase over the parent drug).
Emodin	Tyrosine kinase inhibitor	D-rhamnose	A594, HepG2, OVCAR-3, Hela, K562 and SGC-790 (cell proliferation was inhibited and EM-d-Rha conjugate displayed IC_{50} values in low μmolar ranges).
Paclitaxel	Mitotic inhibitor	Glucose	NCI-H838, MES-SA, HCT-116, and NPC-TW01 (cell proliferation was inhibited the conjugated presented higher cytotoxicity, induced tubulin aggregation and chromosomal condensation compared to paclitaxel).
Doxorubicin	Antitumor antibiotic	Galactose	*In vitro* viability of HepG2 (hepatocarcinoma) and MCF-7 (breast cancer) tumor cells incubated with DOX was higher than that of Gal-DOX. *In vivo* experiments showed that tumor size in Gal-DOX-treated groups was greatly reduced in comparison to the DOX-treated group.
Doxorubicin	Antitumor antibiotic	2-amino-2-deoxy-D-glucose and succinic acid	*In vitro* and *in vivo* studies showed that 2DG-SUC-ADM induced a higher level of apoptosis and higher inhibition rates in MCF-7 and HepG2 tumoral cells than the parent aglycone ADM.

breast, prostate, central nervous system, and ovarian cell lines. The synthesis consists of several chemical steps to perform chlorambucil-based libraries for chemoselective glycosylation. On the basis of this study, the neoglycosides, D-glucuronolactonide and D-threoside were selected as the most potent antitumoral agents. D-threoside glycoside manifested an 8-fold higher efficacy in general, with a respective 12-fold, and 15-fold greater effectiveness in targeting the malignant cell lines HT-29, and HCT-15 (from colorectal adenocarcinomas) over the parent drug. The authors concluded that D-threoside was the most active chlorambucil neoglycoside among the compounds tested. In summary, a novel panel of glycoconjugates were designed and synthesized through the use of common metabolic carbohydrates that are preferentially recognized by cell-membrane receptors, therefore favoring the uptake of the glycodrugs[38]. The specific mechanisms and the receptors or transporters involved in this process remain to be elucidated.

Geldanamycin

Geldanamycin (GE) is a potent anticancer antibiotic that inhibits the heat-shock protein 90 (Hsp90)[39]. This protein is a molecular chaperon involved in the modulation of the activity of various protein kinases. Hsp90 has been found to be 2- to 10-fold more expressed in various human cancer cells than in normal tissues, suggesting the use of that protein as a possible target for cancer therapy[40]. Although

GE has long been recognized as an inhibitor of tumor growth, the potential clinical utility of that agent is hampered by its severe side effects[41,42]. To circumvent this problem, an approach involving a binary chemical step was designed by Cheng *et al*[43] (2005), resulting in a series of glycosyl-GE derivatives. The enzyme-specific activation of these glycosylated prodrugs with galactose and glucose moieties was performed with α-galactosidase and ß-glucosidase, respectively. The effect of the resulting derivatives was evaluated in different cancer-cell lines, including SW620, HT-29, MCF-7, and K-562 leukemia cells. In particular, the glucose-GE exhibited antitumor activity after cleavage of the glucose moiety by the β-glucosidase inside the tumor cells. The anticancer activity of the galactose-GE conjugate increased by 3- to 40-fold when incubated with exogenous galactosidase *in vitro*, but remained inactive in the absence of the added enzyme because, unlike intracellular glucosidases, galactosidases are present in only low or undetectable levels in serum[43]. Consequently, the activation of the galactosyl-GE prodrugs by the exogenous enzyme would appear to be a necessary targeting approach, involving a strategy leading to a dual tactic consisting of the glycosylation of the enzyme as well as the drug, in order to direct the whole system to the target tissue[8,9].

Emodin

Emodin (1,3,8-trihydroxy-6-methyl-anthraquinone) is a natural anthraquinone derivative found in the roots and rhizomes of numerous plants. As a tyrosine-kinase inhibitor, emodin inhibits cell growth in several types of tumor cells[44-46] and regulates the expression of genes involved in cell apoptosis, oncogenesis, cell proliferation, and cancer-cell invasiveness and metastasis[47-49]. The antitumor effects of emodin have been described, but the molecular mechanism has not been fully elucidated. The synthesis and design of emodin conjugated to D-rhamnose (EM-D-Rha), inhibited cell proliferation in a panel of different human-cancer-cell lines including A549 (lung carcinoma), HepG2 (hepatoma), OVCAR-3 (ovarian carcinoma), HeLa (cervical carcinoma) and K-562 (chronic myelogenous leukaemia) and SGC-790 (endocervical adenocarcinoma). EM-d-Rha also manifested lower IC_{50} values and was 10-fold more cytotoxically effective than the aglycone on HepG2 cells, leading to a decrease in mitochondrial transmembrane potential and to an upregulation of the expression of apoptosis elements[50].

Doxorubicin (Adriamycin)

Doxorubicin (DOX), the active compound in the trade drug named adriamycin (ADM), belongs to the anthracycline family, is one of the most powerful and widely used chemotherapeutic drugs, and as such is recognized by the World Health Organization[51]. Unfortunately, apart from the drug's activity as an intercalating agent and topoisomerase-II inhibitor, DOX causes severe toxic effects such as nephrotoxicity, hepatotoxicity, and alopecia, compromising mainly heart tissues and the gastrointestinal tract as a result of the compound's systemic action. In view of the many efforts that have been made order to overcome these limitations, an increase in drug efficiency through the conjugation of DOX to a carbohydrate ligand that can specifically recognize tumoral cells is a promising approach[52,53].

Ma *et al*[54] (2015) conjugated galactose to DOX covalently to form the prodrug Gal-DOX and then evaluated the derivative's tumor-targeting capability in different cancer-cell lines. They found that Gal-Dox treatment increased cell death in HepG2 and MCF-7 cells compared to DOX. These results were the opposite when they treated normal L02 hepatocytes. They proposed that this difference was caused by the high specific binding of Gal to ASGPR1 receptors, which were overexpressed on the surface of HepG2 and MCF-7 tumor cells. Consistent with that conclusion, since those nonmalignant hepatocytes contained lower plasma-membrane levels of ASGPR1, the L02 cells maintained high cell viability, thus suggesting low toxicity of Gal-DOX. As to side effects, Gal-DOX is accumulated in heart tissue to a lesser extent than DOX, thus diminishing myocardial damage. *In vivo* results indicated a reduction in tumor size in the Gal-DOX-treated group compared to the DOX-treated group. Of notable relevance here is that the survival of the Gal-DOX-treated group was 100%, whereas the rate of DOX-treated group was 50%, thus supporting the conclusion that Gal-Dox preferentially targeted the tumor cells. These findings suggest that Gal-DOX is a promising drug for tumor- directed therapy[54]. In another study, ADM was conjugated with 2-amino-2-deoxy-D-glucose and succinic acid (2DG–SUC–ADM). There the investigators found that the ternary derivative was highly specific for tumor cells (MCF-7 and HepG2) *via* the GLUT-1 transporter, while exerting no significant adverse effects on normal cells. The action of the glycoconjugate was also confirmed *in vivo*, thus demonstrating that the glycosylated molecule could specifically target tumoral cells as opposed to the aglycone. These results suggest 2DG–SUC–ADM as a promising drug for targeting cancer cells[55].

Paclitaxel

Paclitaxel is a cytotoxic chemotherapeutic drug, classified as a "plant alkaloid," a "taxane" and an "antimicrotubule agent"[55,56]. The molecular basis for the action of paclitaxel consists in a binding to tubulin subunits, thus disrupting mitosis and causing cell death[57-59]. Despite being widely used in the clinic, this drug has pronoinced side-effects; thus affecting both normal cells and tumors. Another problem with paclitaxel is its poor water solubility. Lin *et al*[60] (2008) designed glycan-based paclitaxel prodrugs, consisting of 2'-paclitaxel conjugated with glucosyl or glucuronyl residues by an ester or an ether linkage. These glycodrugs not only had increased solubility in water compared to the parent compound, but exhibited higher selectivity against targeted cancer cells as well. Glucosyl-paclitaxel displayed the higher cytotoxicity and could induce tubulin aggregation and chromosomal condensation in a tumor-cell line[60]. The cells overexpressing GLUTs favored the uptake of glucose-paclitaxel and facilitated the entrance of the bulky compounds. Therefore, the authors proposed the synthesis of glucoconjugates as an alternative approach for improving the directed delivery of drugs to cancer cells overexpressing GLUTs[60].

5-fluorouracil

5-fluorouracil (5Fu) is part of a group of chemotherapeutic drugs known as antimetabolites. These compounds incorporate into normal macromolecules to produce a slightly different structure that interferes in the metabolism of the cancer cells. 5Fu is one of the most commonly used drugs to treat gastrointestinal and breast cancers, although the agent is mostly used in combination with other drugs like oxaliplatin[61-63].

Davis and coauthors synthesized the prodrugs DOX and 5Fu capped by L-rhamnose and evaluated their use in a lectin-directed–enzyme-activated-prodrug therapy (LEAPT) system. The LEAPT system uses biocatalysts for site-selective drug delivery through the construction of novel glycosylated enzymes and prodrugs. The drugs capped with rhamnose synthesized by Davis and coworkers were released by an α-rhamnosidase, which enzyme is by itself glycosylated with galactose. The glycosylated enzyme specifically targets the ASGPR hepatic-receptors. The biodistribution revealed that the glycosylated enzyme became quickly sequestered to the liver, and to a lower extent to the kidney and bladder. Of essential relevance was that the co-administration of the prodrugs did not interfere in the colocalization of the LEAPT system[8,9]. Finally, the authors demonstrated that the prodrug could be activated in the liver only by the presence of the prelocalized glycosylated enzyme[8].

CARBOHYDRATE-BASED VACCINES

Within glycoscience new approaches are being undertaken for cancer therapy. Glycan-based vaccines have been developed for the specific enhancement of the immune response. The more complex task in formulating a cancer vaccine would be the selection of the appropriate antigen, which molecule should be exclusively expressed by cancer cells. Tumor-associated carbohydrate antigens, ought to be cellular components that are essential for malignant-cell survival in order to prevent the downregulation of the antigen and thus maintain the immune response. Tumor-associated carbohydrate antigens can be divided into two classes: glycoprotein antigens: The Thomsen Nouveau (Tn; GalNAc-a1-O-Ser/Thr), Thomsen-Friendreich (TF), and sialyl-Tn (sTn) linked to the hydroxyl group of serine or threonine residues of proteins and glycolipids[64]. Several studies demonstrated that altered glycosylation and aberrant glycan structures helped tumor cells to circumvent immune surveillance. Since high-affinity T cells recognizing self-antigens are eliminated during development of the central immune system, tumor-associated carbohydrate antigen-directed cancer vaccines face the challenge of activating any remaining low-affinity T cells[65-69].

Glycan-based vaccines may prove to be beneficial because unusual glycan motifs on glycoproteins can lead to vaccines with high specificity[70]. Mucin 1 (MUC1) is an *O*-linked glycan transmembrane protein overexpressed in various tumors—such as lung, breast, pancreas, kidney, ovary, and colon—and has been demonstrated to be aberrantly glycosylated in cancer cells but highly glycosylated in normal cells. Up to the present, most vaccines have targeted nonglycosylated MUC1, although this approach did not prove to be cancer-specific. Thus, targeting cancer-associated glycopeptide epitopes in MUC1 would be a promising alternative possibility[71-73]. New attempts have been made to develop glycosylation-based vaccines that target MUC1. In 2012, Madsen *et al*[74] found that the addition of GalNAc residues to MUC1

to aid antigen uptake and major-histocompatability-complex–class-II presentation for the generation of a potent cancer-specific humoral response. In another investigation, Li *et al*[75] designed and synthesized two linear trivalent glycopeptides of the immune-dominant epitope of MUC1. The antibodies induced by glycosylated-MUC1–based vaccines had a stronger binding than those raised by nonglycosylated MUC1, thus, having the potential to overcome the weak immunogenicity of natural MUC1 glycopeptides[75]. Further studies demonstrated that the antibodies elicited by a vaccine composed of the immunoadjuvant (Pam3CysSK4), a peptide T-helper epitope, and an aberrantly glycosylated MUC1 peptide, were significantly more lytic and more effective in tumor prevention than the unglycosylated control[76]. Other glycan-based vaccines that lead to higher immune responses are presently under development. The most likely to be effective is a vaccine composed of MUC1 glycopeptide in combination with a T-helper peptide, while another uses a combination of MUC1 with the toll-like receptor 2. The last one is composed of MUC1, toll-like receptor 2/9, and a Th peptide[70].

No clinical trials are currently being undertaken on gastrointestinal tumors, although a phase-III clinical trial is being carried out in patients with metastatic breast cancer that involves the glycan-based vaccine, sTn-KLH. The sTn-KLH–vaccine group evidenced an improved survival, relative to the overall survival among the patients treated without the vaccine[77,78].

Many efforts have been made to develop carbohydrate-based vaccines, though until now, none have been approved for clinical use, and many cancer-vaccine candidates have failed in clinical trials. One reason could be the ability of tumor cells to escape from the endogenous immune response or downregulate the immune-target molecules. Nevertheless, an optimization of vaccine formulation in terms of receptor- or antigen-targeting delivery systems may lead to significant improvements in the utilization of currently available carbohydrate antigens so as to open new perspectives for cancer treatment.

PERSPECTIVES

The conjugation of anticancer agents to carbohydrate-ligands that preferentially target tumor cells has resulted in the prediction that several conjugates would prove to have clinical efficacy. Specifically, glycoconjugation offers an improvement in targeting cancer cells, since are many sugar receptors are overexpressed in tumoral cells[79,80] (Figure 1). Furthermore, the addition of a sugar moiety (*e.g.*, glucose, galactose, rhamnose) improves the water solubility and stability of the parent drugs. During recent years, this field has been emerging in translational medicine; but, in closing, we need to stress that glycodrug development is a rigorous process that still requires many steps to determine the true utility of that strategy.

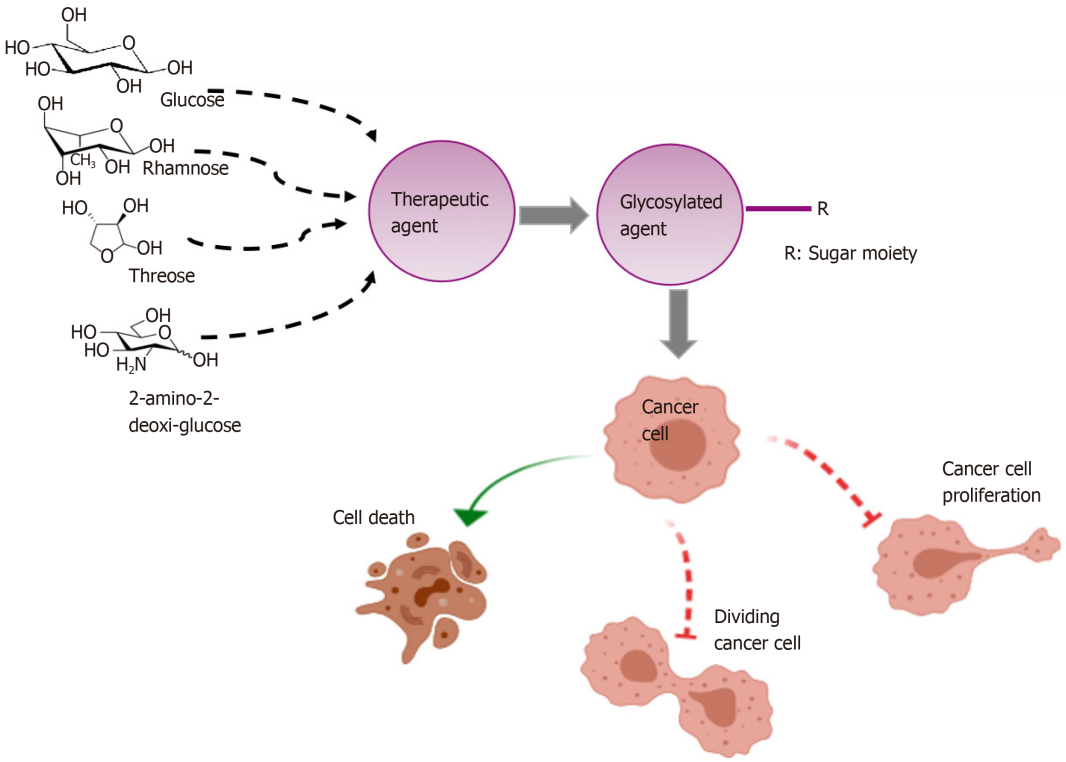

Figure 1 Glycoconjugation strategy. Different sugar moieties can be conjugated to therapeutic agents, with the aim at improving drug efficiency, including antiproliferative activity and growth inhibition, and upregulating cancer-cell death.

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REFERENCES

1 **Cancer Research UK**. General cancer information. Available from: https://www.cancerresearchuk.org/about-cancer/cancer-in-general

2 **Global Burden of Disease Cancer Collaboration**. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, Dicker DJ, Chimed-Orchir O, Dandona R, Dandona L, Fleming T, Forouzanfar MH, Hancock J, Hay RJ, Hunter-Merrill R, Huynh C, Hosgood HD, Johnson CO, Jonas JB, Khubchandani J, Kumar GA, Kutz M, Lan Q, Larson HJ, Liang X, Lim SS, Lopez AD, MacIntyre MF, Marczak L, Marquez N, Mokdad AH, Pinho C, Pourmalek F, Salomon JA, Sanabria JR, Sandar L, Sartorius B, Schwartz SM, Shackelford KA, Shibuya K, Stanaway J, Steiner C, Sun J, Takahashi K, Vollset SE, Vos T, Wagner JA, Wang H, Westerman R, Zeeb H, Zoeckler L, Abd-Allah F, Ahmed MB, Alabed S, Alam NK, Aldhahri SF, Alem G, Alemayohu MA, Ali R, Al-Raddadi R, Amare A, Amoako Y, Artaman A, Asayesh H, Atnafu N, Awasthi A, Saleem HB, Barac A, Bedi N, Bensenor I, Berhane A, Bernabé E, Betsu B, Binagwaho A, Boneya D, Campos-Nonato I, Castañeda-Orjuela C, Catalá-López F, Chiang P, Chibueze C, Chitheer A, Choi JY, Cowie B, Damtew S, das Neves J, Dey S, Dharmaratne S, Dhillon P, Ding E, Driscoll T, Ekwueme D, Endries AY, Farvid M, Farzadfar F, Fernandes J, Fischer F, G/Hiwot TT, Gebru A, Gopalani S, Hailu A, Horino M, Horita N, Husseini A, Huybrechts I, Inoue M, Islami F, Jakovljevic M, James S, Javanbakht M, Jee SH, Kasaeian A, Kedir MS, Khader YS, Khang YH, Kim D, Leigh J, Linn S, Lunevicius R, El Razek HMA, Malekzadeh R, Malta DC, Marcenes W, Markos D, Melaku YA, Meles KG, Mendoza W, Mengiste DT, Meretoja TJ, Miller TR, Mohammad KA, Mohammadi A, Mohammed S, Moradi-Lakeh M, Nagel G, Nand D, Le Nguyen Q, Nolte S, Ogbo FA, Oladimeji KE, Oren E, Pa M, Park EK, Pereira DM, Plass D, Qorbani M, Radfar A, Rafay A, Rahman M, Rana SM, Søreide K, Satpathy M, Sawhney M, Sepanlou SG, Shaikh MA, She J, Shiue I, Shore HR, Shrime MG, So S, Soneji S, Stathopoulou V, Stroumpoulis K, Sufiyan MB, Sykes BL, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tessema GA, Thakur JS, Tran BX, Ukwaja KN, Uzochukwu BSC, Vlassov VV, Weiderpass E, Wubshet Terefe M, Yebyo HG, Yimam HH, Yonemoto N, Younis MZ, Yu C, Zaidi Z, Zaki MES, Zenebe ZM, Murray CJL, Naghavi M. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol* 2017; **3**: 524-548 [PMID: 27918777 DOI: 10.1001/jamaoncol.2016.5688]

3 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018:

GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
4 **Taylor WF**, Jabbarzadeh E. The use of natural products to target cancer stem cells. *Am J Cancer Res* 2017; **7**: 1588-1605 [PMID: 28744407]
5 **Rao D**, Parakrama R, Augustine T, Liu Q, Goel S, Maitra R. Immunotherapeutic advances in gastrointestinal malignancies. *NPJ Precis Oncol* 2019; **3**: 4 [PMID: 30729176 DOI: 10.1038/s41698-018-0076-8]
6 **Skeel RT**. Antineoplastic drugs and biological response modifiers. In: Skeel RT. Handbook of cancer chemotherapy. Philadelpia: Lippincott Williams and Wilkins, 1999: 108-109
7 **Sathornsumetee S**, Rich JN. Molecularly targeted therapy in neuro-oncology. In: Aminoff MJ, Boller F, Swaab DF, editors. Handbook of Clinical Neurology. Elsevier, 2012: 255-278 [PMID: 22230448 DOI: 10.1016/B978-0-444-52138-5.00018-9]
8 **Robinson MA**, Charlton ST, Garnier P, Wang XT, Davis SS, Perkins AC, Frier M, Duncan R, Savage TJ, Wyatt DA, Watson SA, Davis BG. LEAPT: lectin-directed enzyme-activated prodrug therapy. *Proc Natl Acad Sci USA* 2004; **101**: 14527-14532 [PMID: 15448212 DOI: 10.1073/pnas.0303574101]
9 **Garnier P**, Wang XT, Robinson MA, van Kasteren S, Perkins AC, Frier M, Fairbanks AJ, Davis BG. Lectin-directed enzyme activated prodrug therapy (LEAPT): Synthesis and evaluation of rhamnose-capped prodrugs. *J Drug Target* 2010; **18**: 794-802 [PMID: 21047273 DOI: 10.3109/1061186X.2010.529909]
10 **Rogers JC**, Kornfeld S. Hepatic uptake of proteins coupled to fetuin glycopeptide. *Biochem Biophys Res Commun* 1971; **45**: 622-629 [PMID: 5128172 DOI: 10.1016/0006-291x(71)90462-1]
11 **Annibaldi A**, Widmann C. Glucose metabolism in cancer cells. *Curr Opin Clin Nutr Metab Care* 2010; **13**: 466-470 [PMID: 20473153 DOI: 10.1097/MCO.0b013e32833a5577]
12 **Warburg O**. On the origin of cancer cells. *Science* 1956; **123**: 309-314 [PMID: 13298683 DOI: 10.1126/science.123.3191.309]
13 **Gonzalez CD**, Alvarez S, Ropolo A, Rosenzvit C, Bagnes MF, Vaccaro MI. Autophagy, Warburg, and Warburg reverse effects in human cancer. *Biomed Res Int* 2014; **2014**: 926729 [PMID: 25197670 DOI: 10.1155/2014/926729]
14 **Uldry M**, Thorens B. The SLC2 family of facilitated hexose and polyol transporters. *Pflugers Arch* 2004; **447**: 480-489 [PMID: 12750891 DOI: 10.1007/s00424-003-1085-0]
15 **Kunkel M**, Reichert TE, Benz P, Lehr HA, Jeong JH, Wieand S, Bartenstein P, Wagner W, Whiteside TL. Overexpression of Glut-1 and increased glucose metabolism in tumors are associated with a poor prognosis in patients with oral squamous cell carcinoma. *Cancer* 2003; **97**: 1015-1024 [PMID: 12569601 DOI: 10.1002/cncr.11159]
16 **Thorens B**, Mueckler M. Glucose transporters in the 21st Century. *Am J Physiol Endocrinol Metab* 2010; **298**: E141-E145 [PMID: 20009031 DOI: 10.1152/ajpendo.00712.2009]
17 **Carvalho KC**, Cunha IW, Rocha RM, Ayala FR, Cajaíba MM, Begnami MD, Vilela RS, Paiva GR, Andrade RG, Soares FA. GLUT1 expression in malignant tumors and its use as an immunodiagnostic marker. *Clinics (Sao Paulo)* 2011; **66**: 965-972 [PMID: 21808860 DOI: 10.1590/s1807-59322011000600008]
18 **Deng D**, Xu C, Sun P, Wu J, Yan C, Hu M, Yan N. Crystal structure of the human glucose transporter GLUT1. *Nature* 2014; **510**: 121-125 [PMID: 24847886 DOI: 10.1038/nature13306]
19 **Calvaresi EC**, Hergenrother PJ. Glucose conjugation for the specific targeting and treatment of cancer. *Chem Sci* 2013; **4**: 2319-2333 [PMID: 24077675 DOI: 10.1039/C3SC22205E]
20 **Coulibaly FS**, Youan BC. Current Status of Lectin-Based Cancer Diagnosis and Therapy. *AIMS Mol Sci* 2017; **4**: 1–27 [DOI: 10.3934/molsci.2017.1.1]
21 **Cash HL**, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 2006; **313**: 1126-1130 [PMID: 16931762 DOI: 10.1126/science.1127119]
22 **Thomas GB**, Rader LH, Park J, Abezgauz L, Danino D, DeShong P, English DS. Carbohydrate modified catanionic vesicles: probing multivalent binding at the bilayer interface. *J Am Chem Soc* 2009; **131**: 5471-5477 [PMID: 19323555 DOI: 10.1021/ja8076439]
23 **Seeberger PH**, Werz DB. Synthesis and medical applications of oligosaccharides. *Nature* 2007; **446**: 1046-1051 [PMID: 17460666 DOI: 10.1038/nature05819]
24 **Sharon N**, Lis H. History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology* 2004; **14**: 53R-62R [PMID: 15229195 DOI: 10.1093/glycob/cwh122]
25 **Shahzad-ul-Hussan S**, Cai M, Bewley CA. Unprecedented glycosidase activity at a lectin carbohydrate-binding site exemplified by the cyanobacterial lectin MVL. *J Am Chem Soc* 2009; **131**: 16500-16508 [PMID: 19856962 DOI: 10.1021/ja905929c]
26 **Stowell SR**, Arthur CM, Dias-Baruffi M, Rodrigues LC, Gourdine JP, Heimburg-Molinaro J, Ju T, Molinaro RJ, Rivera-Marrero C, Xia B, Smith DF, Cummings RD. Innate immune lectins kill bacteria expressing blood group antigen. *Nat Med* 2010; **16**: 295-301 [PMID: 20154696 DOI: 10.1038/nm.2103]
27 **Lerchen HG**, Baumgarten J, Piel N, Kolb-Bachofen V. Lectin-Mediated Drug Targeting: Discrimination of Carbohydrate-Mediated Cellular Uptake between Tumor and Liver Cells with Neoglycoconjugates Carrying Fucose Epitopes Regioselectively Modified in the 3-Position. *Angew Chem Int Ed Engl* 1999; **38**: 3680-3683 [PMID: 10649323 DOI: 10.1002/(SICI)1521-3773(19991216)38:24<3680::AID-ANIE3680>3.0.CO;2-9]
28 **Lis H**, Sharon N. Lectins: Carbohydrate-Specific Proteins That Mediate Cellular Recognition. *Chem Rev* 1998; **98**: 637-674 [PMID: 11848911 DOI: 10.1021/cr940413g]
29 **Xu L**, Liu X, Li Y, Yin Z, Jin L, Lu L, Qu J, Xiao M. Enzymatic rhamnosylation of anticancer drugs by an α-L-rhamnosidase from Alternaria sp. L1 for cancer-targeting and enzyme-activated prodrug therapy. *Appl Microbiol Biotechnol* 2019; **103**: 7997-8008 [PMID: 31414160 DOI: 10.1007/s00253-019-10011-0]
30 **Wang Y**, Gao J, Gu G, Li G, Cui C, Sun B, Lou H. In situ RBL receptor visualization and its mediated anticancer activity for solasodine rhamnosides. *Chembiochem* 2011; **12**: 2418-2420 [PMID: 21953983 DOI: 10.1002/cbic.201100551]
31 **Stüben J**, Port R, Bertram B, Bollow U, Hull WE, Schaper M, Pohl J, Wiessler M. Pharmacokinetics and whole-body distribution of the new chemotherapeutic agent beta-D-glucosylisophosphoramide mustard and its effects on the incorporation of [methyl-3H]-thymidine in various tissues of the rat. *Cancer Chemother Pharmacol* 1996; **38**: 355-365 [PMID: 8674159 DOI: 10.1007/s002800050495]
32 **Pohl J**, Bertram B, Hilgard P, Nowrousian MR, Stüben J, Wiessler M. D-19575--a sugar-linked isophosphoramide mustard derivative exploiting transmembrane glucose transport. *Cancer Chemother Pharmacol* 1995; **35**: 364-370 [PMID: 7850916 DOI: 10.1007/s002800050248]
33 **Stenn KS**. Metastasizing neoplastic cells. *Am J Dermatopathol* 1979; **1**: 375-376 [PMID: 543531 DOI:

10.1097/00000372-197900140-00017]
34 **Briasoulis E**, Judson I, Pavlidis N, Beale P, Wanders J, Groot Y, Veerman G, Schuessler M, Niebch G, Siamopoulos K, Tzamakou E, Rammou D, Wolf L, Walker R, Hanauske A. Phase I trial of 6-hour infusion of glufosfamide, a new alkylating agent with potentially enhanced selectivity for tumors that overexpress transmembrane glucose transporters: a study of the European Organization for Research and Treatment of Cancer Early Clinical Studies Group. *J Clin Oncol* 2000; **18**: 3535-3544 [PMID: 11032596 DOI: 10.1200/JCO.2000.18.20.3535]
35 **Chiorean EG**, Dragovich T, Hamm J, Barrios CH, Gorini CF, Langmuir VK, Kroll S, Jung DT, Tidmarsh GT, Loehrer PJ. A phase 2 trial of glufosfamide in combination with gemcitabine in chemotherapy-naive pancreatic adenocarcinoma. *Am J Clin Oncol* 2010; **33**: 111-116 [PMID: 19687729 DOI: 10.1097/COC.0b013e3181979204]
36 **Hartley JA**, Bingham JP, Souhami RL. DNA sequence selectivity of guanine-N7 alkylation by nitrogen mustards is preserved in intact cells. *Nucleic Acids Res* 1992; **20**: 3175-3178 [PMID: 1620613 DOI: 10.1093/nar/20.12.3175]
37 **Zhang HJ**, Jinks RN, Wishart AC, Battelle BA, Chamberlain SC, Fahrenbach WH, Kass L. An enzymatically enhanced recording technique for Limulus ventral photoreceptors: physiology, biochemistry, and morphology. *Vis Neurosci* 1994; **11**: 41-52 [PMID: 8011582 DOI: 10.1017/s0952523800011093]
38 **Goff RD**, Thorson JS. Assessment of chemoselective neoglycosylation methods using chlorambucil as a model. *J Med Chem* 2010; **53**: 8129-8139 [PMID: 20973561 DOI: 10.1021/jm101024j]
39 **Neckers L**. Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol Med* 2002; **8**: S55-S61 [PMID: 11927289 DOI: 10.1016/s1471-4914(02)02316-x]
40 **Ferrarini M**, Heltai S, Zocchi MR, Rugarli C. Unusual expression and localization of heat-shock proteins in human tumor cells. *Int J Cancer* 1992; **51**: 613-619 [PMID: 1601523 DOI: 10.1002/ijc.2910510418]
41 **Supko JG**, Hickman RL, Grever MR, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother Pharmacol* 1995; **36**: 305-315 [PMID: 7628050 DOI: 10.1007/BF00689048]
42 **Sausville EA**. Combining cytotoxics and 17-allylamino, 17-demethoxygeldanamycin: sequence and tumor biology matters. Commentary re: P. Münster *et al*., Modulation of Hsp90 function by ansamycins sensitizes breast cancer cells to chemotherapy-induced apoptosis in an RB- and schedule-dependent manner. Clin. Cancer Res., 7: 2228-2236, 2001. *Clin Cancer Res* 2001; **7**: 2155-2158 [PMID: 11489788]
43 **Cheng H**, Cao X, Xian M, Fang L, Cai TB, Ji JJ, Tunac JB, Sun D, Wang PG. Synthesis and enzyme-specific activation of carbohydrate-geldanamycin conjugates with potent anticancer activity. *J Med Chem* 2005; **48**: 645-652 [PMID: 15658879 DOI: 10.1021/jm049693a]
44 **Chen YC**, Shen SC, Lee WR, Hsu FL, Lin HY, Ko CH, Tseng SW. Emodin induces apoptosis in human promyeloleukemic HL-60 cells accompanied by activation of caspase 3 cascade but independent of reactive oxygen species production. *Biochem Pharmacol* 2002; **64**: 1713-1724 [PMID: 12445860 DOI: 10.1016/s0006-2952(02)01386-2]
45 **Su YT**, Chang HL, Shyue SK, Hsu SL. Emodin induces apoptosis in human lung adenocarcinoma cells through a reactive oxygen species-dependent mitochondrial signaling pathway. *Biochem Pharmacol* 2005; **70**: 229-241 [PMID: 15941563 DOI: 10.1016/j.bcp.2005.04.026]
46 **Srinivas G**, Anto RJ, Srinivas P, Vidhyalakshmi S, Senan VP, Karunagaran D. Emodin induces apoptosis of human cervical cancer cells through poly(ADP-ribose) polymerase cleavage and activation of caspase-9. *Eur J Pharmacol* 2003; **473**: 117-125 [PMID: 12892828 DOI: 10.1016/s0014-2999(03)01976-9]
47 **Huang Q**, Shen HM, Shui G, Wenk MR, Ong CN. Emodin inhibits tumor cell adhesion through disruption of the membrane lipid Raft-associated integrin signaling pathway. *Cancer Res* 2006; **66**: 5807-5815 [PMID: 16740720 DOI: 10.1158/0008-5472.CAN-06-0077]
48 **Kwak HJ**, Park MJ, Park CM, Moon SI, Yoo DH, Lee HC, Lee SH, Kim MS, Lee HW, Shin WS, Park IC, Rhee CH, Hong SI. Emodin inhibits vascular endothelial growth factor-A-induced angiogenesis by blocking receptor-2 (KDR/Flk-1) phosphorylation. *Int J Cancer* 2006; **118**: 2711-2720 [PMID: 16388516 DOI: 10.1002/ijc.21641]
49 **Cha TL**, Qiu L, Chen CT, Wen Y, Hung MC. Emodin down-regulates androgen receptor and inhibits prostate cancer cell growth. *Cancer Res* 2005; **65**: 2287-2295 [PMID: 15781642 DOI: 10.1158/0008-5472.CAN-04-3250]
50 **Xing JY**, Song GP, Deng JP, Jiang LZ, Xiong P, Yang BJ, Liu SS. Antitumor effects and mechanism of novel emodin rhamnoside derivatives against human cancer cells in vitro. *PloS One* 2015; **10**: e0144781 [PMID: 26682731 DOI: 10.1371/journal.pone.0144781]
51 **The WHOQOL Group**. The World Health Organization quality of life assessment (WHOQOL): position paper from the World Health Organization. *Soc Sci Med* 1995; **41**: 1403-1409 [PMID: 8560308 DOI: 10.1016/0277-9536(95)00112-K]
52 **Mahato R**, Tai W, Cheng K. Prodrugs for improving tumor targetability and efficiency. *Adv Drug Deliv Rev* 2011; **63**: 659-670 [PMID: 21333700 DOI: 10.1016/j.addr.2011.02.002]
53 **de Bono JS**, Ashworth A. Translating cancer research into targeted therapeutics. *Nature* 2010; **467**: 543-549 [PMID: 20882008 DOI: 10.1038/nature09339]
54 **Ma Y**, Chen H, Su S, Wang T, Zhang C, Fida G, Cui S, Zhao J, Gu Y. Galactose as Broad Ligand for Multiple Tumor Imaging and Therapy. *J Cancer* 2015; **6**: 658-670 [PMID: 26078797 DOI: 10.7150/jca.11647]
55 **Cao J**, Cui S, Li S, Du C, Tian J, Wan S, Qian Z, Gu Y, Chen WR, Wang G. Targeted cancer therapy with a 2-deoxyglucose-based adriamycin complex. *Cancer Res* 2013; **73**: 1362-1373 [PMID: 23396585 DOI: 10.1158/0008-5472.CAN-12-2072]
56 **Rooseboom M**, Commandeur JN, Vermeulen NP. Enzyme-catalyzed activation of anticancer prodrugs. *Pharmacol Rev* 2004; **56**: 53-102 [PMID: 15001663 DOI: 10.1124/pr.56.1.3]
57 **Abraham S**, Guo F, Li LS, Rader C, Liu C, Barbas CF, Lerner RA, Sinha SC. Synthesis of the next-generation therapeutic antibodies that combine cell targeting and antibody-catalyzed prodrug activation. *Proc Natl Acad Sci USA* 2007; **104**: 5584-5589 [PMID: 17372220 DOI: 10.1073/pnas.0700223104]
58 **Chatterjee SK**, Zetter BR. Cancer biomarkers: knowing the present and predicting the future. *Future Oncol* 2005; **1**: 37-50 [PMID: 16555974 DOI: 10.1517/14796694.1.1.37]
59 **de Graaf M**, Boven E, Scheeren HW, Haisma HJ, Pinedo HM. Beta-glucuronidase-mediated drug release. *Curr Pharm Des* 2002; **8**: 1391-1403 [PMID: 12052215 DOI: 10.2174/1381612023394485]
60 **Wu J**, Liu Q, Lee RJ. A folate receptor-targeted liposomal formulation for paclitaxel. *Int J Pharm* 2006; **316**: 148-153 [PMID: 16564654 DOI: 10.1016/j.ijpharm.2006.02.027]

61 **Lin YS**, Tungpradit R, Sinchaikul S, An FM, Liu DZ, Phutrakul S, Chen ST. Targeting the delivery of glycan-based paclitaxel prodrugs to cancer cells via glucose transporters. *J Med Chem* 2008; **51**: 7428-7441 [PMID: 19053781 DOI: 10.1021/jm8006257]

62 **Longley DB**, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; **3**: 330-338 [PMID: 12724731 DOI: 10.1038/nrc1074]

63 **Johnston PG**, Kaye S. Capecitabine: a novel agent for the treatment of solid tumors. *Anticancer Drugs* 2001; **12**: 639-646 [PMID: 11604550 DOI: 10.1097/00001813-200109000-00001]

64 **Feng D**, Shaikh AS, Wang F. Recent Advance in Tumor-associated Carbohydrate Antigens (TACAs)-based Antitumor Vaccines. *ACS Chem Biol* 2016; **11**: 850-863 [PMID: 26895482 DOI: 10.1021/acschembio.6b00084]

65 **Hollingsworth RE**, Jansen K. Turning the corner on therapeutic cancer vaccines. *NPJ Vaccines* 2019; **4**: 7 [PMID: 30774998 DOI: 10.1038/s41541-019-0103-y]

66 **Carrascal MA**, Severino PF, Guadalupe Cabral M, Silva M, Ferreira JA, Calais F, Quinto H, Pen C, Ligeiro D, Santos LL, Dall'Olio F, Videira PA. Sialyl Tn-expressing bladder cancer cells induce a tolerogenic phenotype in innate and adaptive immune cells. *Mol Oncol* 2014; **8**: 753-765 [PMID: 24656965 DOI: 10.1016/j.molonc.2014.02.008]

67 **Pashov A**, Monzavi-Karbassi B, Kieber-Emmons T. Immune surveillance and immunotherapy: lessons from carbohydrate mimotopes. *Vaccine* 2009; **27**: 3405-3415 [PMID: 19200843 DOI: 10.1016/j.vaccine.2009.01.074]

68 **Pashov A**, Monzavi-Karbassi B, Raghava GP, Kieber-Emmons T. Bridging innate and adaptive antitumor immunity targeting glycans. *J Biomed Biotechnol* 2010; **2010**: 354068 [PMID: 20617150 DOI: 10.1155/2010/354068]

69 **Pinho SS**, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 2015; **15**: 540-555 [PMID: 26289314 DOI: 10.1038/nrc3982]

70 **Ho WL**, Hsu WM, Huang MC, Kadomatsu K, Nakagawara A. Protein glycosylation in cancers and its potential therapeutic applications in neuroblastoma. *J Hematol Oncol* 2016; **9**: 100 [PMID: 27686492 DOI: 10.1186/s13045-016-0334-6]

71 **Sørensen AL**, Reis CA, Tarp MA, Mandel U, Ramachandran K, Sankaranarayanan V, Schwientek T, Graham R, Taylor-Papadimitriou J, Hollingsworth MA, Burchell J, Clausen H. Chemoenzymatically synthesized multimeric Tn/STn MUC1 glycopeptides elicit cancer-specific anti-MUC1 antibody responses and override tolerance. *Glycobiology* 2006; **16**: 96-107 [PMID: 16207894 DOI: 10.1093/glycob/cwj044]

72 **Tarp MA**, Sørensen AL, Mandel U, Paulsen H, Burchell J, Taylor-Papadimitriou J, Clausen H. Identification of a novel cancer-specific immunodominant glycopeptide epitope in the MUC1 tandem repeat. *Glycobiology* 2007; **17**: 197-209 [PMID: 17050588 DOI: 10.1093/glycob/cwl061]

73 **Ryan SO**, Vlad AM, Islam K, Gariépy J, Finn OJ. Tumor-associated MUC1 glycopeptide epitopes are not subject to self-tolerance and improve responses to MUC1 peptide epitopes in MUC1 transgenic mice. *Biol Chem* 2009; **390**: 611-618 [PMID: 19426130 DOI: 10.1515/BC.2009.070]

74 **Madsen CB**, Petersen C, Lavrsen K, Harndahl M, Buus S, Clausen H, Pedersen AE, Wandall HH. Cancer associated aberrant protein O-glycosylation can modify antigen processing and immune response. *PLoS One* 2012; **7**: e50139 [PMID: 23189185 DOI: 10.1371/journal.pone.0050139]

75 **Li M**, Yu F, Yao C, Wang PG, Liu Y, Zhao W. Synthetic and immunological studies on trimeric MUC1 immunodominant motif antigen-based anti-cancer vaccine candidates. *Org Biomol Chem* 2018; **16**: 993-999 [PMID: 29345713 DOI: 10.1039/c7ob02976d]

76 **Lakshminarayanan V**, Thompson P, Wolfert MA, Buskas T, Bradley JM, Pathangey LB, Madsen CS, Cohen PA, Gendler SJ, Boons GJ. Immune recognition of tumor-associated mucin MUC1 is achieved by a fully synthetic aberrantly glycosylated MUC1 tripartite vaccine. *Proc Natl Acad Sci USA* 2012; **109**: 261-266 [PMID: 22171012 DOI: 10.1073/pnas.1115166109]

77 **Miles D**, Papazisis K. Rationale for the clinical development of STn-KLH (Theratope) and anti-MUC-1 vaccines in breast cancer. *Clin Breast Cancer* 2003; **3** Suppl 4: S134-S138 [PMID: 12620150 DOI: 10.3816/cbc.2003.s.002]

78 **Rodríguez E**, Schetters STT, van Kooyk Y. The tumour glyco-code as a novel immune checkpoint for immunotherapy. *Nat Rev Immunol* 2018; **18**: 204-211 [PMID: 29398707 DOI: 10.1038/nri.2018.3]

79 **Altenberg B**, Greulich KO. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. *Genomics* 2004; **84**: 1014-1020 [PMID: 15533718 DOI: 10.1016/j.ygeno.2004.08.010]

80 **Medina RA**, Owen GI. Glucose transporters: expression, regulation and cancer. *Biol Res* 2002; **35**: 9-26 [PMID: 12125211 DOI: 10.4067/s0716-97602002000100004]

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REVIEW

Roles of cell fusion, hybridization and polyploid cell formation in cancer metastasis

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Abstract

Cell-cell fusion is a normal biological process playing essential roles in organ formation and tissue differentiation, repair and regeneration. Through cell fusion somatic cells undergo rapid nuclear reprogramming and epigenetic modifications to form hybrid cells with new genetic and phenotypic properties at a rate exceeding that achievable by random mutations. Factors that stimulate cell fusion are inflammation and hypoxia. Fusion of cancer cells with non-neoplastic cells facilitates several malignancy-related cell phenotypes, *e.g.*, reprogramming of somatic cell into induced pluripotent stem cells and epithelial to mesenchymal transition. There is now considerable *in vitro, in vivo* and clinical evidence that fusion of cancer cells with motile leucocytes such as macrophages plays a major role in cancer metastasis. Of the many changes in cancer cells after hybridizing with leucocytes, it is notable that hybrids acquire resistance to chemo- and radiation therapy. One phenomenon that has been largely overlooked yet plays a

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role in these processes is polyploidization. Regardless of the mechanism of polyploid cell formation, it happens in response to genotoxic stresses and enhances a cancer cell's ability to survive. Here we summarize the recent progress in research of cell fusion and with a focus on an important role for polyploid cells in cancer metastasis. In addition, we discuss the clinical evidence and the importance of cell fusion and polyploidization in solid tumors.

Key words: Cell fusion; Hybrid formation; Polyploidization; Macrophage; Cancer progression; Oncologic treatment resistance

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Core tip: Cell fusion is a normal biological process involved in organ formation and tissue repair. Through cell fusion, somatic cells undergo nuclear reprogramming and epigenetic modifications to form hybrid cells with new properties. Fusion of cancer cells with macrophages plays a major role in cancer metastasis and results in resistance to chemo- and radiation therapy. Cell fusion might be a potential target for the development of new antitumor therapies through macrophage depletion in tumour stroma and prevention of cell fusion and post-hybridization events involving chemotaxis and cell migration to lymph nodes and distant metastases.

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INTRODUCTION

Cell fusion is a common biological process that produces viable cells and plays a major role in mammalian tissue development and differentiation. Cell fusion is essential during embryogenesis and morphogenesis, *e.g.*, when trophoblasts in placenta fuse to form syncytiotrophoblasts, in developing of skeletal muscle that arises from the fusion of mesodermal cells[1], and when osteoclasts and multinucleated giant cells are generated from the fusion of macrophages[2,3]. Cell fusion also plays a role in tissue repair and regeneration, *e.g.*, in liver, heart and intestine[4-6]. Through cell fusion, somatic cells undergo nuclear reprogramming and epigenetic modifications to form pluripotent hybrid cells. Cell fusion can result in rapid modifications of the genetic and epigenetic programs of cells, generating cells with new properties at a rate exceeding that achievable by random mutations[6-8].

Cell fusion may result in two forms of hybrids: Heterokaryons or synkaryons. When bi- or multinucleated hybrids are generated (heterokaryons), the parental genomes are located in different nuclei, segregated from one another. These hybrids are capable of cell divisions resulting in daughter cells expressing both parental sets of chromosomes in a single nucleus[9,10]. Hybrids with parental genomes mixed in a single nucleus are called synkaryons[11-14]. In culture, fusion events depend on the cell density, the cell ratio of the parental populations and their microenvironment[15].

CELL FUSION, POLYPLOIDIZATION AND CANCER

Polyploid cells contain more than two basic sets of chromosomes. Polyploidy is a natural phenomenon that contributes to tissue differentiation, normal organogenesis and tissue repair[16,17]. It appears that most polyploid cells in mammals are formed through cell fusion[18], but also through abnormal cell division such as endoreplication, endomitosis and failed cytokinesis after completion of mitosis[19-21]. The formation of multinucleated fusion hybrids may allow genetic complementation capable of rescuing loss of gene function after chemotherapeutic or radiation induced DNA damage[22-24]. Formation of polyploid cells with a selective advantage may serve as a cell survival mechanism[25].

In 1911, a German professor, Otto Aichel, proposed that fusion of cancer cells with

leucocytes produces hybrids with a metastatic phenotype. A century later, this prescient insight has proven to be correct in many studies. Indeed, leucocyte-cancer cell fusion causes cellular reprogramming and generates new clones of hybrids at least some of which acquire the ability for chemotactic migration by combining the epigenetic program of the leucocyte with the uncontrolled cell division of the cancer cell[8,26,27] (Figure 1). Tumour cells are fusogenic and fuse with other cancer cells and non-neoplastic cells in the tumour stroma. Spontaneous fusion between cancer cells is a well-documented phenomenon in solid tumors and generates heterogeneous subpopulations of tumor cells[28-31]. Several studies have shown that fusion of cancer cells with non-neoplastic cells facilitates several malignancy-related cell phenotypes, *e.g.*, reprogramming of somatic cell into induced pluripotent stem cells and epithelial to mesenchymal transition (EMT) (Figure 1). These processes also produce cellular polyploidy.

Polyploid giant cancer cells (PGCCs) are cells with multiple nuclei or a single giant nucleus containing multiple sets of chromosomes. Compared to regular diploid cells, PGCCs can be 10 to 20 times larger in size and have tetraploid or greater (≥ 4C) DNA content[32]. The mechanism leading to formation of PGCC may have similarities to wound repair and tissue regeneration, each of which utilizes or depends on cell fusion and endomitotic and endoreplication mechanisms. Regardless of the mechanism of polyploid cells formation, it happens in response to genotoxic stresses such as those occurring in hypoxic/necrotic regions of the tumor and during chemo- and radiotherapy. Polyploidy then enhances a cancer cell's ability to survive. Even though the formation of hybrids between cancer cells and bone marrow derived cells (BMDCs) most likely occurs stochastically[26], disease progression and standard of care treatments such as chemo- and radiotherapy, can act as driving forces that may increase the frequency of hybridization events[33,34]. The basis of this increase may be due to increased inflammation and/or an aberrant wound and damage response mechanism leading to the formation of PGCC[35,36].

Aneuploidy is a hallmark of cancer and is proposed to have a fundamental role during tumour initiation and progression. Approximately 90% of solid tumors and 75% of hematopoietic malignancies have abnormal chromosome numbers[37,38]. Centrosome aberrations are suggested as one mechanism that causes the formation of aneuploid genomes. An important evolutionary feature of polyploid cancer cells is the generation of aneuploid clones during the reversal of the polyploid state, which is a chaotic process with many genomic translocations, amplifications and deletions occurring during creation of progeny[39]. The presence of centrosome aberrations in polyploid cancer cells suggests that cell fusion and the formation of polyploid cancer cells may be strong contributors to aneuploidy[40,41].

Cell fusion and cancer-stem cells

Cancer stem cells (CSCs) are subpopulations of tumour cells with stem cell-like traits. These traits include high plasticity and the capacity for self-renewal through asymmetric division. CSCs sustain tumourigenesis and generate diverse progeny with the ability to remain dormant while demonstrating resistance to conventional cancer therapeutics[42]. The stem cell theory in cancer, however, is debated due to controversy about evidence supporting the origins of CSCs, their differentiation and dedifferentiation, genetic heterogeneity, symmetric and asymmetric concepts of cellular division, and clonal evolution[43,44].

Cell fusion could be a mechanism to generate CSCs (Figure 2). Gauck *et al*[45] showed that spontaneous fusion of human breast epithelial cells and human breast cancer cells can give rise to hybrid cells that possess CSC properties with significantly increased colony forming capacity compared to the maternal epithelial cells. In a polyethylene glycol mediated fusion experiment, Flasza *et al*[46] demonstrated that murine P19 and human NTERA2/D1 embryonal carcinoma hybrid cells displayed heterogeneity in cellular morphology and gene expression. The hybrids expressed stemness factors octamer-binding transcription factor 4, homebox protein Nanog and Sex Determining Region Y-Box 2, indicating the activation of endogenous human markers of pluripotency. In another example, spontaneously formed heterotypic hybrids between mesenchymal stem cells and lung cancer cells expressed the stem cell marker prominin-1, which was increased 30-fold in hybrids cells compared to their maternal lung cancer cells. The hybrids also exhibited increased expression of the transcription factors octamer-binding transcription factor 4, Aldehyde dehydrogenase 1, B-lymphoma Mo-MLV insertion region 1, and Sex Determining Region Y-Box 2, suggestive of a stem cell-like phenotype[47].

Cell fusion causes chromosomal instability, tumour heterogeneity and DNA exchange

Most malignant tumors are polyclonal. Clonal heterogeneity may be caused by

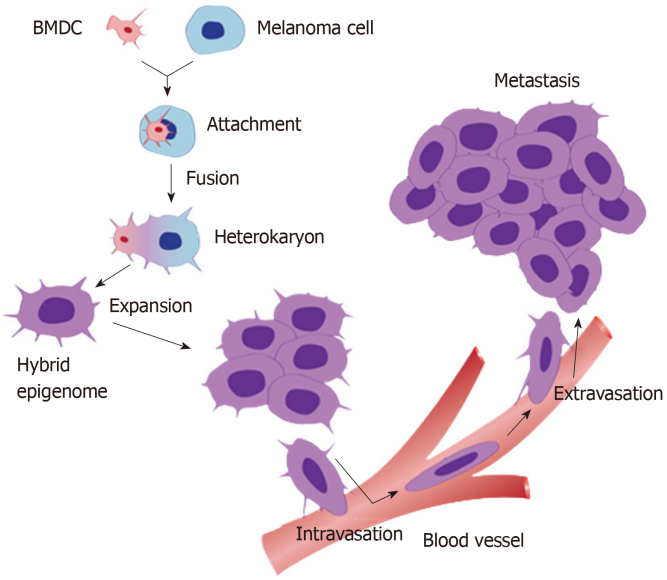

Figure 1 A schematic diagram of the process of cell fusion, hybrid formation and metastasis. A motile bone marrow derived cells (red) such as a macrophage or stem cell is drawn to a cancer cell (blue). The outer cell membranes of the two cells become attached. Fusion occurs with the formation of a bi-nucleated heterokaryon having a nucleus from each of the fusion partners. The heterokaryon goes through genomic hybridization creating a cancer cell-bone marrow derived cells hybrid with co-expressed epigenomes, conferring deregulated cell division and metastatic competence to the hybrid. BMDC: Bone marrow derived cell.

oncogenic mutations in single cells, epigenetic modulations[48], and cell fusion[49]. Chromothripsis, massive genomic rearrangements[50], is defined as a single catastrophic event in the development of cancer[51]. In oral squamous cell carcinoma cell lines in culture, irradiation predisposes to cells chromothripsis. The finding of fragmented DNA in aneuploid hybrid cells is an indicator of chromothripsis[52].

Cell fusion may be homotypic between the same types of cells in the tissue or heterotypic between different cell types like epithelial cells and macrophages. Heterotypic fusion can cause multiple changes in gene expression profiles in the resultant hybrids[10]. Clonal heterogeneity patterns within primary tumors are often similar to those of distant metastases with similar gene expression profiles. Using a Cre-loxP model system, Searles *et al*[53] showed that Cre transfer occurred between cancer and non-cancer cells both in cell cultures and in mice. The rapid transfer of Cre could not be explained by extracellular vesicles but rather by cell fusion.

Cell fusion, cancer and EMT

In order to form metastases, tumour cells need to navigate through a series of obstacles that require a variety of cellular functions and abilities that were absent in the transformed cells of origin. The functions include an invasive escape from the tumour and intravasation into blood or lymphatic vessels. All steps of the metastatic cascade require an ability to overcome the induction of cell death. To escape the circulation, tumour cells need to adhere to the vessel wall and undergo extravasation into other tissues. Once in the tissue, cell growth is required to form metastasis. One mechanism put forth to explain the changes required to perform these functions is EMT. This model explains how neoplastic cells may gain a migratory and invasive phenotype allowing them to escape from the primary tumour. Many studies have identified a subset of embryonic-like transcription factors, such as zinc finger protein SNAI1 and basic helix-loop-helix factor Twist, that form the basis of a gene expression program that drives the transitional change of the phenotype. An alternative mechanism is that cancer-mesenchymal cell fusions generate hybrids that gain the genetic, phenotypic and functional properties of both maternal cells. Xu *et al*[54] showed in an *in vivo* non-obese diabetic/severe combined immunodeficiency mouse model that fusion of mesenchymal stem cells with non-small cell lung cancer cells results in hybrids that express both epithelial and mesenchymal markers with increased migratory and invasive capabilities compared to their maternal cancer cells.

In studies by Zhang *et al*[55], analysis of polyploidy giant cells (referred to by the authors as "PGCC") in colorectal cancer revealed a strong association with the presence of lymph node metastasis. Potentially the PGCC were responsible for metastasis as a subset of "budding" daughter cells showed a greater migratory and

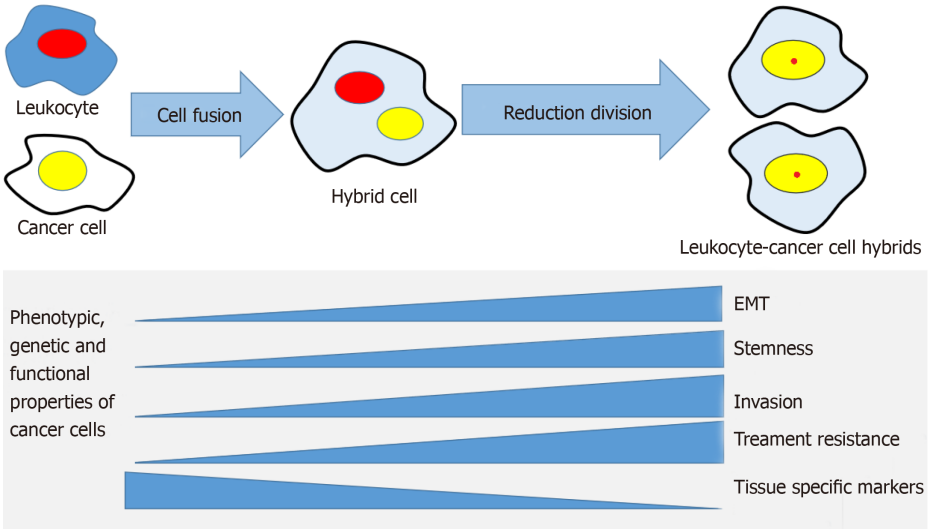

Figure 2 The cell fusion theory in relation to cancer progression mechanisms. A cancer cell and a leukocyte form a hybrid that acquires genetic, phenotypic and functional properties from both maternal cells. The hybrid cells develop properties associated with cancer metastasis, such as epithelial mesenchymal transition, stemness, invasiveness, treatment resistance and may lose some of the maternal cancer cell's tissue-specific phenotype. EMT: Epithelial to mesenchymal transition.

invasive phenotype and expressed the EMT-related proteins Twist and Snail. Similarly, PGCCs induced by the hypoxia mimetic cobalt chloride were capable of generating small diploid cell progeny that also displayed higher levels of EMT related protein expression including vimentin and N-cadherin. These daughter cells had a more invasive phenotype compared to the parental cell type. Importantly, the authors showed that patient samples from breast tumours and metastasis had an increased number of PGCCs with vimentin and N-cadherin expression compared with patient breast tumour samples with no metastasis[56], indicating a higher metastatic potential of the progeny from the PGCCs.

PGCCs and mitochondrial function

PGCCs form under a diverse set of stimuli as they are found within and adjacent to necrotic regions of tumours, driven by conditions of hypoxia, nutrient deprivation (starvation) and low pH. Individually these stimuli have been shown to induce PGCC *in vitro*. Importantly, oxidative stress is the common feature that links these stimuli. Chemotherapies and radiotherapy which can dramatically induce the transformation to PGCCs also generate oxidative stress generated through excess reactive oxygen species (ROS). Mitochondria are the main generator of ROS and may also act as "sensors" to trigger the PGCC transformation which form and exist under conditions of high oxidative stress[57-60]. Roh *et al*[57] showed that PGCCs have increased mitochondrial content as well as elevated levels of ROS. The authors found a correlation between the high ROS levels characteristic of the polyploid cells together with taxol resistance that could be reversed with the use of antioxidants. Similarly, the ROS-producing agent plumbagin induced features of PGCCs on prostate cancer cells, in line with the idea that polyploidy provides a survival advantage to cells that are exposed to high levels of ROS. The authors suggested that besides impacting cancer cell resistance to therapy, the polyploid state could also contribute to the generation of CSCs in response to stress[59]. In the tumour microenvironment, where growth conditions deteriorate and oxidative stress increases with progression[61], PGCC are induced at higher rates[33,62]. Even the number of circulating hybrid cancer cells is increased as shown in liquid biopsies[63-66].

How cell fusion promotes tumour progression

Cell fusion contributes to tumour progression not only through alteration of the composition and biology of tumour cells *per se*, but also by modifying the tumour microenvironment. Hybridization of cancer cells with *e.g.*, BMDCs, generates hybrids with a significantly faster growth rate *in vivo*[67,68] and enhanced abilities of colony formation[68], cell migration and invasion[47]. Thus, cell fusion alters the biological behaviour of a tumour through the development of new metastatic tumour cell sets with growth-promoting properties, contributing to enhanced tumour growth and metastasis[69]. Similar observations have been reported in several other studies over the past three decades[14,70-73].

Cell fusion also contributes to neo-angiogenesis and thus facilitates hematogenic

and lymphatogenic intravasation of tumour cells. Recently, Shen *et al*[74] demonstrated that dendritic cells can fuse with endothelial progenitor cells and generate hybrids with a significantly faster growth rate, while also producing a greater number of micro-vessels compared to their maternal endothelial progenitor cell. Busund *et al*[75] showed that *in vivo* growth of cultured tumours consisting of Metha-A sarcoma cell/macrophage hybrids had a significantly higher intratumoural microvessel density and maturation compared to tumours from maternal Metha A sarcoma cells alone. The hybrids released significantly higher amounts of angiogenic peptides, such vascular endothelial growth factor (VEGF), compared to both maternal macrophages and cancer cells. In renal tissues of individuals with gender-mismatched transplants who had transplant rejection and chronic inflammation, Kerjaschki *et al*[76] provided clinical evidence that BMDCs, presumably macrophages, function as progenitors of lymphatic endothelial cells, and contribute to lymphangiogenesis by incorporating into the new lymphatic vessel. Moreover, the macrophage might differentiate to VEGF producing cells that drive the division of endothelial cells[77,78].

MACROPHAGES AS FUSION PARTNERS IN CANCER

Macrophages are a heterogeneous population of cells derived from monocytes. During embryogenesis, they appear first in the yolk sac, then in the liver, and finally in bone marrow. Large populations of tissue macrophages exist in the small intestine, liver (Kupffer cells), and lungs. Blood monocytes arise in the marrow from precursor cells (monoblasts) and enter inflamed or infected tissues, where they may mature into macrophages and increase the resident macrophage populations. Monocytes may also mature into dendritic cells presenting antigens to T cells. Fusion is an important function of macrophages and results in the formation of osteoclasts and multinucleated giant cells[3].

Macrophages show two different polarization states, M1 and M2, in response to different signals in the microenvironment. M1 macrophages are pro-inflammatory and characterized by the release of inflammatory cytokines and microbicidal/tumoricidal activity. M2-macrophages have an immunosuppressive phenotype and are polarized by anti-inflammatory molecules such as Interleukin (IL)-4, IL-13, and IL-10, apoptotic cells, and immune complexes. M2 macrophages release anti-inflammatory cytokines and have scavenging potentials as well as supporting angiogenesis and tissue repair. Monocyte/macrophage cells are important for tumour cell migration, invasion and metastasis. Tumour associated macrophages (TAM) represent the M2-type and promote tumor progression[79-83].

Monocytes are actively recruited to the tumour stroma, and high infiltration of TAMs in many tumour types correlate with lymph node involvement and distant metastases[84]. Inhibition of macrophage infiltration in tumours may suppress metastasis[85,86]. The clinical significance of macrophage infiltration in tumour stroma, however, is still controversial. High infiltration of TAMs is correlated to poor prognosis in breast, prostatic, ovarian and cervical carcinoma[87]. In colorectal cancer, there are conflicting data of the clinical significance of macrophage infiltration, but several studies show that low macrophage density in tumour stroma is associated with an unfavourable prognosis[87-89].

TAMs contribute to angiogenesis, lymphangiogenesis and tumour progression by expressing pro-angiogenic growth factors such as matrix metalloproteinase-12, IL-1, VEGF, and IL-8. Clinical studies have shown that increased infiltration of TAMs in solid tumours is associated with high micro-vessel density and poor prognosis. These data are particularly strong for hormone-dependent cancers, such as breast cancer[87,90].

INFLAMMATION AND CELL FUSION

The mechanisms causing cell fusion and polyploidization in tumours have close similarities to wound repair and tissue regeneration in non-transformed tissues. Under normal conditions, fusion events are exceptionally rare but increase dramatically in pathological conditions such as after tissue injury and during inflammation. Nygren *et al*[34] showed that BMDC contribute to the formation of stable and reprogrammed fusion-hybrids in liver, heart and myocardium during tissue repair. Interestingly, the authors observed that despite an attraction of fusogenic blood cells to these tissues following injury-induced inflammation, the cell fusion was restricted to a subset of cells implicated in syncytia formation during development. Johansson *et al*[91] reported in a parabiotic experimental mouse-model that chronic inflammation, induced by idiopathic ulcerative dermatitis and autoimmune

encephalitis, could increase myelomonocytic cells in peripheral blood and consequently significantly increase (10–100-fold higher) heterokaryon formation of BMDCs with Purkinje neurons.

HYPOXIA/TISSUE STARVATION AND CELL FUSION

The tumour microenvironment is usually characterized by poor vascularization, resulting in hypoxia and deficient access to vital nutrients and elimination of metabolic by-products. Necrosis is common in the deeper parts of the tumour. Despite these seemingly harsh conditions, cells that subsist in this environment have been linked to a more malignant phenotype with stem cell-like properties[92,93].

As mentioned above, cell fusion is induced by hypoxia and apoptosis. Noubissi *et al*[94] used bimolecular fluorescence complementation to detect *in vitro* spontaneous fusion events between co-cultured multipotent stem/stromal cells (mSSC) and either human breast epithelial cells (MCF-10a) or breast cancer cell lines (T47D, MDA-MB-231 and MCF-7). The co-cultures were grown in hypoxic condition (2% O_2 compared to standard tissue culture conditions of 21% O_2) and it was found that hypoxia stimulated a significant increase in fusion between the mSSC and the T47D or MCF-7 non-metastatic breast cancer cells. Hypoxia had, however, no significant impact on the fusion ability of MDA-MB-231 metastatic cancer cells. The authors suggest that hypoxia might promote fusion of non-metastatic cancer cells and therefore enables metastasis, an event that perhaps is not advantageous for the already metastatic MDA-MB-231 cells. It was found also that apoptosis was enhanced by hypoxia in T47D and MCF7 non-metastatic cancer cells. The fusion events were increased if the cell co-cultures were supplemented with apoptotic T47D cells in both normoxic and hypoxic conditions, indicating that cell fusion could be stimulated by apoptosis independently of hypoxia. Melzer *et al*[95] reported similar *in vitro* observations and found that cell fusion events varied dramatically between benign or malignant breast cells when used as fusion partners for mSSC. Co-cultures of mSSC with MCF-10a revealed increased fusion events up to 10-fold (> 2%) compared with co-cultures with MDA-MB-231 (0.2%) or with MCF-7 (0.1%). In line with the observations that inflammation can induce cell fusion, the author stimulated the cell co-cultures with pro-inflammatory cytokine TNF-α and found significant hybrid cell formation compared to non-treated cells.

Using different cancer cell lines treated with the hypoxia mimetic cobalt chloride to induce polyploidization, Zhang *et al*[55] showed that the formation of PGCCs could occur either through cell-fusion or endoreplication. The cells had increased size, developed enlarged nuclei and could survive for extended periods of time, while cells with normal morphology could be selectively eliminated. The PGCCs displayed CSC markers and properties and could therefore be potentially more tumourigenic[96].

CLINICAL SIGNIFICANCE OF CELL FUSION AND POLYPLOIDIZATION IN CANCER

PGCCs are found in most cancers and correlate to poor survival[97-100]. Reports of mono and multinucleated cancer cells can be found in the literature dating back to the early 20th century[101-103]. PGCCs are also described as "multi-nucleated giant cells", "tumour budding", "micropapillary", and "osteoclast-like giant cells". Across many cancer types, when present in high numbers, PGCCs of epithelial-origin show highly malignant characteristics, including chemo-resistance, with short patient survival and are designated with World Health Organization sub-classification status. These rare cancers include, giant cell carcinoma of the lung (a sub-classification of the large cell/sarcomatoid carcinomas of the lung) and pleomorphic carcinoma of the lung[104-106]. Although rare, these cancers provide important insights into the highly malignant nature of the PGCCs. Patient survival is typically worse than in those with the non-PGCC cancer component due to poor or refractory responses to standard treatments, short relapse intervals and enhanced metastatic spread[32,56,97,99].

PGCC formation has been demonstrated to increase during tumour progression[55,56,99,100], as late stage cancers have elevated numbers of PGCCs. It might be predicted that through increased PGCC formation in late stage disease, increased numbers of heterotypic fusion events also occur. Recent studies on liquid biopsies show the presence of hybrid-like expression in multinucleated giant cells. This phenotype is shown to correlate with malignancy and poor survival[66,107-109].

Several studies have shown that after standard of care treatments treatments with radiation[33,35,110], chemotherapy[40] or targeted therapy[111], PGCC can be formed using

cell fusion as a mechanism. The PGCCs can overcome the treatment-induced damage and produce progeny that are resistant to treatments to which the cancer cells are normally sensitive. This has been demonstrated for both irradiation and chemotherapy and may be an important factor in disease relapse and patient outcome[40,112-114].

PGCCs have been shown in many tumour types to be associated with increased therapy resistance and poor survival[104,106,115]. For example, in a syngeneic rat tumour model, Puig *et al*[116] showed that despite an initial shrinkage of tumours in response to cisplatin treatment, tumour cells enter a latent phase from which they are eventually able to escape and resume growth. These cells survived for weeks and while some eventually died, others were able to undergo a reversal of polyploidization giving rise to new colonies of diploid cells, which were more resistant to cytotoxic drugs and were responsible for tumour relapse. Additionally, a single large multinucleated cell isolated from the murine fibrosarcoma cell line UV-2237 could produce tumours in mice. These large multinucleated cells were also more resistant to doxorubicin suggesting that they could be driving the relapse[117].

Clinical evidence of cell fusion

It has been well demonstrated that leucocyte-cancer cell fusion produces hybrid cells that express genetic and phenotypic characteristics of both maternal cells[71,118]. Clinically, it is difficult to detect or genetically confirm fusion events because the genetic content of maternal cells and any hybrids have the same origin. The expression of tissue specific proteins by tumour cells and other fusion cell partners, like TAMs, may, however, constitute surrogate markers that could be used for detecting the presence of fusion events in tumour tissue from clinical patient material.

CD163 is a macrophage specific trans-membrane scavenger receptor and its presence indicates that the cells have an M2-macrophage phenotype[119]. Macrophage traits in cancer cells, exemplified by CD163 expression, have been reported for several types of tumours, *e.g*., renal cell[120], breast[121], colorectal[122,123] and bladder[124,125] cancers. Based on the cell fusion theory, the macrophage phenotype in cancer cells is suggested to be caused by fusion between TAMs and tumour cells[71,126,127].

In an *in vitro* model, Shabo *et al*[126] showed that cancer cells did not acquire a macrophage phenotype by paracrine interaction between macrophages and MCF-7 breast cancer cells. In contrast, macrophage/MCF-7 hybrids (generated *via* spontaneous cell fusion) expressed macrophage-like markers, CD163 and the pan-leucocyte marker CD45. The hybrids also showed genetic characteristics from both parent cells. Powel *et al*[71,128] provided *in vivo* evidence of fusion between circulating BMDCs and cancer cells during tumourigenesis, demonstrating that macrophages were cellular partners in this process. Silk *et al*[118] showed similar *in vivo* characteristics in human intestinal epithelium. These studies clearly support the many observations that macrophage traits in cancer cells are explained by fusion between tumour cells and TAMs[4,67,75].

Shabo *et al*[121] reported that CD163 expression by tumour cells in breast cancer was seen in 48% of a cohort of 133 female patients. The patients with CD163-positive tumours had reduced recurrence-free survival times. Further, CD163 expression by cancer cells was more common in advanced cancers. Epithelial cells in normal breast tissue showed no expression of CD163. In a similar study, the same research group reported CD163 expression by tumour cells in 23% of 139 patients with rectal cancer. Again, CD163 expression was not seen in any of the non-cancerous areas from adjacent or distant rectal tissue. CD163 expression by tumour cells was associated with earlier local recurrence and shorter cancer specific survival, and inversely correlated to apoptosis. Notably, the expression of CD163 by cancer cells was more common (31%) in tumours from patients treated with preoperative radiotherapy compared to those not treated (17%).

The expression of macrophage traits by cancer cells was proportional to intra-tumoural macrophage density indicating that increased recruitment and infiltration of macrophages in tumour tissue may result in higher rates of fusion between macrophages and cancer cells in tumour stroma[122,124]. The frequency of cell fusion events *in vivo* was as high as 1% in experimental tumour models[27]. The fusion efficiency increased proportionally to the presence of inflammation[91] and the metastatic potential of tumour cells[129]. Macrophage-cancer cell hybrids are generated spontaneously in cultured breast cancer cells at an average rate of 2 % and are able to survive cell culture for several weeks[126]. Del Monte demonstrated that one gram of tumour contained some 10^8 tumour cells[130]. Based on this calculation, a rate of 1%-2 % frequency of cell fusion events means that each gram of solid tumour may contain as many as 1-2 million hybrids. Although the proportion of hybrids may be small in relation to the total tumour mass, the spontaneity of cell fusion and the survival of the hybrids may generate deadly derivative clones with important clinical implications.

Garvin *et al*[131] calculated the proportion of cancer cells expressing CD163 in 83 patients with breast cancer and found that the number of CD163-positive cells in all breast tumors studies averaged 9% (range 0%-41%). CD163-expression of > 15% of cancer cells was associated with breast cancer-related death (P = 0.02). The authors also reported that the mean number of cancer cells expressing CD163 was positively associated with mitotic index supporting a connection between fusion events and the density of TAMs seen in tumours. It is likely that the plasticity of reprogrammed cellular phenotypes originating in hybrids may form both dominating as well as volatile clones in the tumour environment.

Through cell fusion cancer cells acquire new phenotypes but may also lose other traits that are specific for their tissue origin, a process known as de-differentiation. These traits are essential in the clinical assessment, *e.g*., the estrogen receptor (ER) pathway is involved in cell growth and regulation of breast cancer cells. Moreover, estrogen is a potent breast mitogen and ER-inhibitors and estrogen-producing enzymes (aromatases) are well-established, effective therapies[132]. ER is down-regulated in progeny cells generated by fusion between macrophages and breast cancer cells[133]. In immuno-histochemical studies of biopsies, macrophage traits in cancer cells (indicating fusion events) was associated with ER-negative tumours[121,131]. These observations have clinical relevance as down-regulation of ER in breast cancer cells will change the pathologic staging and the treatment options for the patients.

Cell fusion pathways as diagnostic and therapeutic targets

Clinical investigations of tumour biology with a focus on cell fusion as an underlying mechanism are limited, probably because this theory has been difficult to investigate and has not been firmly established as other topics, such as the cancer mutation theory. In light of findings on the role of cell fusion in tumour biology reported over the past 30 years, we believe that cell fusion pathways might constitute a target in cancer diagnostics and treatment. As discussed previously, cell fusion contributes to tumour progression by generating new cancer cell clones with enhanced metastatic properties. Morphologically, hybrid cells have similar appearance to their maternal cancer cells. Hence, the malignant potential of tumours might be underestimated if hybrid cells are not detected during clinical histopathological assessment. For example, Busund *et al*[75] showed that *in vivo* tumours consisting of Metha-A sarcoma/macrophage hybrids had the similar histopathological morphology as tumours consisting of maternal Metha-A sarcoma cells. The tumours consisting of hybrid cells had however greater growth rate, metastatic ability and vascular density; tumour characteristics that have prognostic significance in a clinical context.

Consistent with *in vivo* findings, the expression of macrophage traits as surrogate marker for macrophage-cancer cell fusion[4,71,118,134] by cancer cells in clinical tumour material is associated with advanced tumour stage and poor prognosis[120-125,135,136], indicating the importance of identifying and validating histopathological markers, such as macrophage-specific marker CD163, to detect fusion events in clinical tumour material. Accumulating evidence suggests that cell fusion results in the development of stem cell properties and resistance to oncological treatment[33,115,127,137-140]. Cell fusion may have a predictive value in cancer treatment and it should be verified by clinical investigations.

Cell fusion might constitute a therapy target in cancer and can be counteracted by several strategies. Tentatively, inhibition of the cell fusion process or infiltration of cancer cell fusion partners, such as macrophages, might be possible treatment targets. Inhibition of macrophage infiltration into tumour stroma might reduce the frequency of macrophage-cancer cell fusion. Macrophage depletion might also reduce other macrophage related tumour promoting mechanism such as neo-angiogenesis. Although these data are not related to macrophage-cancer cell fusion, several studies show that macrophage depletion in tumour stroma is associated with inhibition of tumour progression[141-144]. For example, Griesmann *et al*[145] showed in an experimental mouse model that treatment with liposomal clondronate decreased macrophage infiltration in several organs and resulted in significant reduction of liver and pulmonary metastasis of pancreatic cancer, independently of the presence of an endogenous primary tumour.

RATIONALE

Using histochemical markers along with genetic analyses it is now clear that cell fusion and hybrid formation are associated with metastasis and poor patient survival. There is an association of polyploidy, produced by leucocyte-cancer cell fusion, with therapy resistance. We may glimpse the engine that drives metastasis (Figures 1 and

2). This information opens many potential targets for the development of new therapies: (1) Inhibition of the fusion process itself regarding events such as membrane attachment and heterokaryon formation; (2) Inhibition of the hybridization processes involving integration of parental fusion partner genes into hybrid genomes; and (3) Prevention of post-hybridization events involving activation of genes that control cell migration, chemotaxis, intravasation, extravasation, and migration to lymph nodes and distant metastases.

CONCLUSION

Cell fusion is a normal biological process that is essential during embryogenesis and morphogenesis. Accumulating evidence indicates that fusion between leukocytes and cancer cells occur in solid tumors and may contribute to tumor progression. These data provide new insights into the role of leukocytes, such as macrophages, in tumor biology and cell fusion as a potential mechanism in tumor metastasis and the development of resistance to oncologic treatment.

REFERENCES

1 **Camargo FD**, Green R, Capetanaki Y, Jackson KA, Goodell MA. Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nat Med* 2003; **9**: 1520-1527 [PMID: 14625546 DOI: 10.1038/nm963]

2 **Paululat A**, Breuer S, Renkawitz-Pohl R. Determination and development of the larval muscle pattern in Drosophila melanogaster. *Cell Tissue Res* 1999; **296**: 151-160 [PMID: 10199975 DOI: 10.1007/s004410051276]

3 **Vignery A**. Macrophage fusion: the making of osteoclasts and giant cells. *J Exp Med* 2005; **202**: 337-340 [PMID: 16061722 DOI: 10.1084/jem.20051123]

4 **Rizvi AZ**, Swain JR, Davies PS, Bailey AS, Decker AD, Willenbring H, Grompe M, Fleming WH, Wong MH. Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. *Proc Natl Acad Sci USA* 2006; **103**: 6321-6325 [PMID: 16606845 DOI: 10.1073/pnas.0508593103]

5 **Vassilopoulos G**, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature* 2003; **422**: 901-904 [PMID: 12665833 DOI: 10.1038/nature01539]

6 **Nygren JM**, Jovinge S, Breitbach M, Säwén P, Röll W, Hescheler J, Taneera J, Fleischmann BK, Jacobsen SE. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med* 2004; **10**: 494-501 [PMID: 15107841 DOI: 10.1038/nm1040]

7 **Alvarez-Dolado M**, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 2003; **425**: 968-973 [PMID: 14555960 DOI: 10.1038/nature02069]

8 **Terada N**, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 2002; **416**: 542-545 [PMID: 11932747 DOI: 10.1038/nature730]

9 **Pereira CF**, Terranova R, Ryan NK, Santos J, Morris KJ, Cui W, Merkenschlager M, Fisher AG. Heterokaryon-based reprogramming of human B lymphocytes for pluripotency requires Oct4 but not Sox2. *PLoS Genet* 2008; **4**: e1000170 [PMID: 18773085 DOI: 10.1371/journal.pgen.1000170]

10 **Piccolo FM**, Pereira CF, Cantone I, Brown K, Tsubouchi T, Soza-Ried J, Merkenschlager M, Fisher AG. Using heterokaryons to understand pluripotency and reprogramming. *Philos Trans R Soc Lond B Biol Sci* 2011; **366**: 2260-2265 [PMID: 21727131 DOI: 10.1098/rstb.2011.0004]

11 **Mortensen K**, Lichtenberg J, Thomsen PD, Larsson LI. Spontaneous fusion between cancer cells and endothelial cells. *Cell Mol Life Sci* 2004; **61**: 2125-2131 [PMID: 15316661 DOI: 10.1007/s00018-004-4200-2]

12 **Vassilopoulos G**, Russell DW. Cell fusion: an alternative to stem cell plasticity and its therapeutic implications. *Curr Opin Genet Dev* 2003; **13**: 480-485 [PMID: 14550412 DOI: 10.1016/s0959-437x(03)00110-2]

13 **Wong C**, Stearns T. Centrosome number is controlled by a centrosome-intrinsic block to reduplication. *Nat Cell Biol* 2003; **5**: 539-544 [PMID: 12766773 DOI: 10.1038/ncb993]

14 **Larizza L**, Schirrmacher V, Pflüger E. Acquisition of high metastatic capacity after in vitro fusion of a nonmetastatic tumor line with a bone marrow-derived macrophage. *J Exp Med* 1984; **160**: 1579-1584 [PMID: 6491605 DOI: 10.1084/jem.160.5.1579]

15 **Ogle BM**, Cascalho M, Platt JL. Biological implications of cell fusion. *Nat Rev Mol Cell Biol* 2005; **6**: 567-575 [PMID: 15957005 DOI: 10.1038/nrm1678]

16 **Losick VP**, Fox DT, Spradling AC. Polyploidization and cell fusion contribute to wound healing in the adult Drosophila epithelium. *Curr Biol* 2013; **23**: 2224-2232 [PMID: 24184101 DOI: 10.1016/j.cub.2013.09.029]

17 **Tamori Y**, Deng WM. Tissue repair through cell competition and compensatory cellular hypertrophy in postmitotic epithelia. *Dev Cell* 2013; **25**: 350-363 [PMID: 23685249 DOI: 10.1016/j.devcel.2013.04.013]

18 **Raslova H**, Kauffmann A, Sekkaï D, Ripoche H, Larbret F, Robert T, Tronik Le Roux D, Kroemer G, Debili N, Dessen P, Lazar V, Vainchenker W. Interrelation between polyploidization and megakaryocyte differentiation: a gene profiling approach. *Blood* 2007; **109**: 3225-3234 [PMID: 17170127 DOI: 10.1182/blood-2006-07-037838]

19 **Li Z**, Lu X, Gao Y, Liu S, Tao M, Xiao H, Qiao Y, Zhang Y and Luo J. Polyploidization and epigenetics. *Chinese Science Bulletin* 2011; **56**: 245-252 [DOI: 10.1007/s11434-010-4290-1]

20 **Miyamoto T**. Regulators of osteoclast differentiation and cell-cell fusion. *Keio J Med* 2011; **60**: 101-105 [PMID: 22200633 DOI: 10.2302/kjm.60.101]

21 **Orr-Weaver TL**. When bigger is better: the role of polyploidy in organogenesis. *Trends Genet* 2015; **31**: 307-315 [PMID: 25921783 DOI: 10.1016/j.tig.2015.03.011]
22 **Duncan AW**, Hickey RD, Paulk NK, Culberson AJ, Olson SB, Finegold MJ, Grompe M. Ploidy reductions in murine fusion-derived hepatocytes. *PLoS Genet* 2009; **5**: e1000385 [PMID: 19229314 DOI: 10.1371/journal.pgen.1000385]
23 **Willenbring H**, Bailey AS, Foster M, Akkari Y, Dorrell C, Olson S, Finegold M, Fleming WH, Grompe M. Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. *Nat Med* 2004; **10**: 744-748 [PMID: 15195088 DOI: 10.1038/nm1062]
24 **Wang X**, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 2003; **422**: 897-901 [PMID: 12665832 DOI: 10.1038/nature01531]
25 **Anatskaya OV**, Vinogradov AE. Genome multiplication as adaptation to tissue survival: evidence from gene expression in mammalian heart and liver. *Genomics* 2007; **89**: 70-80 [PMID: 17029690 DOI: 10.1016/j.ygeno.2006.08.014]
26 **Pawelek JM**, Chakraborty AK. Fusion of tumour cells with bone marrow-derived cells: a unifying explanation for metastasis. *Nat Rev Cancer* 2008; **8**: 377-386 [PMID: 18385683 DOI: 10.1038/nrc2371]
27 **Duelli D**, Lazebnik Y. Cell fusion: a hidden enemy? *Cancer Cell* 2003; **3**: 445-448 [PMID: 12781362 DOI: 10.1016/s1535-6108(03)00114-4]
28 **Yang JY**, Ha SA, Yang YS, Kim JW. p-Glycoprotein ABCB5 and YB-1 expression plays a role in increased heterogeneity of breast cancer cells: correlations with cell fusion and doxorubicin resistance. *BMC Cancer* 2010; **10**: 388 [PMID: 20649952 DOI: 10.1186/1471-2407-10-388]
29 **Ozel C**, Seidel J, Meyer-Staeckling S, Brandt BH, Niggemann B, Zänker KS, Dittmar T. Hybrid cells derived from breast epithelial cell/breast cancer cell fusion events show a differential RAF-AKT crosstalk. *Cell Commun Signal* 2012; **10**: 10 [PMID: 22487193 DOI: 10.1186/1478-811X-10-10]
30 **Wang R**, Chen S, Li C, Ng KT, Kong CW, Cheng J, Cheng SH, Li RA, Lo CM, Man K, Sun D. Fusion with stem cell makes the hepatocellular carcinoma cells similar to liver tumor-initiating cells. *BMC Cancer* 2016; **16**: 56 [PMID: 26846780 DOI: 10.1186/s12885-016-2094-7]
31 **Wakeling WF**, Greetham J, Bennett DC. Efficient spontaneous fusion between some co-cultured cells, especially murine melanoma cells. *Cell Biol Int* 1994; **18**: 207-210 [PMID: 8019495 DOI: 10.1006/cbir.1994.1063]
32 **Lv H**, Shi Y, Zhang L, Zhang D, Liu G, Yang Z, Li Y, Fei F, Zhang S. Polyploid giant cancer cells with budding and the expression of cyclin E, S-phase kinase-associated protein 2, stathmin associated with the grading and metastasis in serous ovarian tumor. *BMC Cancer* 2014; **14**: 576 [PMID: 25106448 DOI: 10.1186/1471-2407-14-576]
33 **Kaur E**, Rajendra J, Jadhav S, Shridhar E, Goda JS, Moiyadi A, Dutt S. Radiation-induced homotypic cell fusions of innately resistant glioblastoma cells mediate their sustained survival and recurrence. *Carcinogenesis* 2015; **36**: 685-695 [PMID: 25863126 DOI: 10.1093/carcin/bgv050]
34 **Nygren JM**, Liuba K, Breitbach M, Stott S, Thorén L, Roell W, Geisen C, Sasse P, Kirik D, Björklund A, Nerlov C, Fleischmann BK, Jovinge S, Jacobsen SE. Myeloid and lymphoid contribution to non-haematopoietic lineages through irradiation-induced heterotypic cell fusion. *Nat Cell Biol* 2008; **10**: 584-592 [PMID: 18425115 DOI: 10.1038/ncb1721]
35 **Illidge TM**, Cragg MS, Fringes B, Olive P, Erenpreisa JA. Polyploid giant cells provide a survival mechanism for p53 mutant cells after DNA damage. *Cell Biol Int* 2000; **24**: 621-633 [PMID: 10964452 DOI: 10.1006/cbir.2000.0557]
36 **Erenpreisa J**, Ivanov A, Wheatley SP, Kosmacek EA, Ianzini F, Anisimov AP, Mackey M, Davis PJ, Plakhins G, Illidge TM. Endopolyploidy in irradiated p53-deficient tumour cell lines: persistence of cell division activity in giant cells expressing Aurora-B kinase. *Cell Biol Int* 2008; **32**: 1044-1056 [PMID: 18602486 DOI: 10.1016/j.cellbi.2008.06.003]
37 **Weaver BA**, Cleveland DW. Does aneuploidy cause cancer? *Curr Opin Cell Biol* 2006; **18**: 658-667 [PMID: 17046232 DOI: 10.1016/j.ceb.2006.10.002]
38 **Duesberg P**, McCormack A. Immortality of cancers: a consequence of inherent karyotypic variations and selections for autonomy. *Cell Cycle* 2013; **12**: 783-802 [PMID: 23388461 DOI: 10.4161/cc.23720]
39 **Liu J**. The dualistic origin of human tumors. *Semin Cancer Biol* 2018; **53**: 1-16 [PMID: 30040989 DOI: 10.1016/j.semcancer.2018.07.004]
40 **Mittal K**, Donthamsetty S, Kaur R, Yang C, Gupta MV, Reid MD, Choi DH, Rida PCG, Aneja R. Multinucleated polyploidy drives resistance to Docetaxel chemotherapy in prostate cancer. *Br J Cancer* 2017; **116**: 1186-1194 [PMID: 28334734 DOI: 10.1038/bjc.2017.78]
41 **Nigg EA**. Centrosome aberrations: cause or consequence of cancer progression? *Nat Rev Cancer* 2002; **2**: 815-825 [PMID: 12415252 DOI: 10.1038/nrc924]
42 **Bu Y**, Cao D. The origin of cancer stem cells. *Front Biosci (Schol Ed)* 2012; **4**: 819-830 [PMID: 22202093 DOI: 10.2741/s302]
43 **LaBarge MA**. The difficulty of targeting cancer stem cell niches. *Clin Cancer Res* 2010; **16**: 3121-3129 [PMID: 20530700 DOI: 10.1158/1078-0432.CCR-09-2933]
44 **Singh AK**, Arya RK, Maheshwari S, Singh A, Meena S, Pandey P, Dormond O, Datta D. Tumor heterogeneity and cancer stem cell paradigm: updates in concept, controversies and clinical relevance. *Int J Cancer* 2015; **136**: 1991-2000 [PMID: 24615680 DOI: 10.1002/ijc.28804]
45 **Gauck D**, Keil S, Niggemann B, Zänker KS, Dittmar T. Hybrid clone cells derived from human breast epithelial cells and human breast cancer cells exhibit properties of cancer stem/initiating cells. *BMC Cancer* 2017; **17**: 515 [PMID: 28768501 DOI: 10.1186/s12885-017-3509-9]
46 **Flasza M**, Shering AF, Smith K, Andrews PW, Talley P, Johnson PA. Reprogramming in inter-species embryonal carcinoma-somatic cell hybrids induces expression of pluripotency and differentiation markers. *Cloning Stem Cells* 2003; **5**: 339-354 [PMID: 14733752 DOI: 10.1089/153623003772032844]
47 **Zhang LN**, Kong CF, Zhao D, Cong XL, Wang SS, Ma L, Huang YH. Fusion with mesenchymal stem cells differentially affects tumorigenic and metastatic abilities of lung cancer cells. *J Cell Physiol* 2019; **234**: 3570-3582 [PMID: 30417342 DOI: 10.1002/jcp.27011]
48 **Duelli D**, Lazebnik Y. Cell-to-cell fusion as a link between viruses and cancer. *Nat Rev Cancer* 2007; **7**: 968-976 [PMID: 18034186 DOI: 10.1038/nrc2272]
49 **Gast CE**, Silk AD, Zarour L, Riegler L, Burkhart JG, Gustafson KT, Parappilly MS, Roh-Johnson M, Goodman JR, Olson B, Schmidt M, Swain JR, Davies PS, Shasthri V, Iizuka S, Flynn P, Watson S, Korkola J, Courtneidge SA, Fischer JM, Jaboin J, Billingsley KG, Lopez CD, Burchard J, Gray J, Coussens LM, Sheppard BC, Wong MH. Cell fusion potentiates tumor heterogeneity and reveals

circulating hybrid cells that correlate with stage and survival. *Sci Adv* 2018; **4**: eaat7828 [PMID: 30214939 DOI: 10.1126/sciadv.aat7828]

50 **Kloosterman WP**, Hoogstraat M, Paling O, Tavakoli-Yaraki M, Renkens I, Vermaat JS, van Roosmalen MJ, van Lieshout S, Nijman IJ, Roessingh W, van 't Slot R, van de Belt J, Guryev V, Koudijs M, Voest E, Cuppen E. Chromothripsis is a common mechanism driving genomic rearrangements in primary and metastatic colorectal cancer. *Genome Biol* 2011; **12**: R103 [PMID: 22014273 DOI: 10.1186/gb-2011-12-10-r103]

51 **Stephens PJ**, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, Pleasance ED, Lau KW, Beare D, Stebbings LA, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal S, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Quail MA, Burton J, Swerdlow H, Carter NP, Morsberger LA, Iacobuzio-Donahue C, Follows GA, Green AR, Flanagan AM, Stratton MR, Futreal PA, Campbell PJ. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell* 2011; **144**: 27-40 [PMID: 21215367 DOI: 10.1016/j.cell.2010.11.055]

52 **Morishita M**, Muramatsu T, Suto Y, Hirai M, Konishi T, Hayashi S, Shigemizu D, Tsunoda T, Moriyama K, Inazawa J. Chromothripsis-like chromosomal rearrangements induced by ionizing radiation using proton microbeam irradiation system. *Oncotarget* 2016; **7**: 10182-10192 [PMID: 26862731 DOI: 10.18632/oncotarget.7186]

53 **Searles SC**, Santosa EK, Bui JD. Cell-cell fusion as a mechanism of DNA exchange in cancer. *Oncotarget* 2018; **9**: 6156-6173 [PMID: 29464062 DOI: 10.18632/oncotarget.23715]

54 **Xu MH**, Gao X, Luo D, Zhou XD, Xiong W, Liu GX. EMT and acquisition of stem cell-like properties are involved in spontaneous formation of tumorigenic hybrids between lung cancer and bone marrow-derived mesenchymal stem cells. *PLoS One* 2014; **9**: e87893 [PMID: 24516569 DOI: 10.1371/journal.pone.0087893]

55 **Zhang D**, Yang X, Yang Z, Fei F, Li S, Qu J, Zhang M, Li Y, Zhang X, Zhang S. Daughter Cells and Erythroid Cells Budding from PGCCs and Their Clinicopathological Significances in Colorectal Cancer. *J Cancer* 2017; **8**: 469-478 [PMID: 28261349 DOI: 10.7150/jca.17012]

56 **Fei F**, Zhang D, Yang Z, Wang S, Wang X, Wu Z, Wu Q, Zhang S. The number of polyploid giant cancer cells and epithelial-mesenchymal transition-related proteins are associated with invasion and metastasis in human breast cancer. *J Exp Clin Cancer Res* 2015; **34**: 158 [PMID: 26702618 DOI: 10.1186/s13046-015-0277-8]

57 **Roh M**, van der Meer R, Abdulkadir SA. Tumorigenic polyploid cells contain elevated ROS and ARE selectively targeted by antioxidant treatment. *J Cell Physiol* 2012; **227**: 801-812 [PMID: 21503880 DOI: 10.1002/jcp.22793]

58 **Gorla GR**, Malhi H, Gupta S. Polyploidy associated with oxidative injury attenuates proliferative potential of cells. *J Cell Sci* 2001; **114** (Pt 16): 2943-2951 [PMID: 11686298]

59 **Balvan J**, Gumulec J, Raudenska M, Krizova A, Stepka P, Babula P, Kizek R, Adam V, Masarik M. Oxidative Stress Resistance in Metastatic Prostate Cancer: Renewal by Self-Eating. *PLoS One* 2015; **10**: e0145016 [PMID: 26671576 DOI: 10.1371/journal.pone.0145016]

60 **Zekri A**, Mesbahi Y, Ghanizadeh-Vesali S, Alimoghaddam K, Ghavamzadeh A, Ghaffari SH. Reactive oxygen species generation and increase in mitochondrial copy number: new insight into the potential mechanism of cytotoxicity induced by aurora kinase inhibitor, AZD1152-HQPA. *Anticancer Drugs* 2017; **28**: 841-851 [PMID: 28639950 DOI: 10.1097/CAD.0000000000000523]

61 **Liou GY**, Döppler H, DelGiorno KE, Zhang L, Leitges M, Crawford HC, Murphy MP, Storz P. Mutant KRas-Induced Mitochondrial Oxidative Stress in Acinar Cells Upregulates EGFR Signaling to Drive Formation of Pancreatic Precancerous Lesions. *Cell Rep* 2016; **14**: 2325-2336 [PMID: 26947075 DOI: 10.1016/j.celrep.2016.02.029]

62 **Espejel S**, Romero R, Alvarez-Buylla A. Radiation damage increases Purkinje neuron heterokaryons in neonatal cerebellum. *Ann Neurol* 2009; **66**: 100-109 [PMID: 19670439 DOI: 10.1002/ana.21670]

63 **Adams DL**, Stefansson S, Haudenschild C, Martin SS, Charpentier M, Chumsri S, Cristofanilli M, Tang CM, Alpaugh RK. Cytometric characterization of circulating tumor cells captured by microfiltration and their correlation to the CellSearch(®) CTC test. *Cytometry A* 2015; **87**: 137-144 [PMID: 25515318 DOI: 10.1002/cyto.a.22613]

64 **Adams DL**, Zhu P, Makarova OV, Martin SS, Charpentier M, Chumsri S, Li S, Amstutz P, Tang CM. The systematic study of circulating tumor cell isolation using lithographic microfilters. *RSC Adv* 2014; **9**: 4334-4342 [PMID: 25614802 DOI: 10.1039/C3RA46839A]

65 **Adams DL**, Adams DK, Stefansson S, Haudenschild C, Martin SS, Charpentier M, Chumsri S, Cristofanilli M, Tang CM, Alpaugh RK. Mitosis in circulating tumor cells stratifies highly aggressive breast carcinomas. *Breast Cancer Res* 2016; **18**: 44 [PMID: 27142282 DOI: 10.1186/s13058-016-0706-4]

66 **Adams DL**, Adams DK, Alpaugh RK, Cristofanilli M, Martin SS, Chumsri S, Tang CM, Marks JR. Circulating Cancer-Associated Macrophage-Like Cells Differentiate Malignant Breast Cancer and Benign Breast Conditions. *Cancer Epidemiol Biomarkers Prev* 2016; **25**: 1037-1042 [PMID: 27197300 DOI: 10.1158/1055-9965.EPI-15-1221]

67 **Busund LT**, Killie MK, Bartnes K, Seljelid R. Spontaneously formed tumorigenic hybrids of Meth A sarcoma cells and macrophages in vivo. *Int J Cancer* 2003; **106**: 153-159 [PMID: 12800188 DOI: 10.1002/ijc.11210]

68 **Zhang LN**, Huang YH, Zhao L. Fusion of macrophages promotes breast cancer cell proliferation, migration and invasion through activating epithelial-mesenchymal transition and Wnt/β-catenin signaling pathway. *Arch Biochem Biophys* 2019; **676**: 108137 [PMID: 31605677 DOI: 10.1016/j.abb.2019.108137]

69 **Pawelek JM**, Chakraborty AK. The cancer cell--leukocyte fusion theory of metastasis. *Adv Cancer Res* 2008; **101**: 397-444 [PMID: 19055949 DOI: 10.1016/S0065-230X(08)00410-7]

70 **De Baetselier P**, Roos E, Brys L, Remels L, Gobert M, Dekegel D, Segal S, Feldman M. Nonmetastatic tumor cells acquire metastatic properties following somatic hybridization with normal cells. *Cancer Metastasis Rev* 1984; **3**: 5-24 [PMID: 6370419 DOI: 10.1007/bf00047690]

71 **Powell AE**, Anderson EC, Davies PS, Silk AD, Pelz C, Impey S, Wong MH. Fusion between Intestinal epithelial cells and macrophages in a cancer context results in nuclear reprogramming. *Cancer Res* 2011; **71**: 1497-1505 [PMID: 21303980 DOI: 10.1158/0008-5472.CAN-10-3223]

72 **Rachkovsky M**, Sodi S, Chakraborty A, Avissar Y, Bolognia J, McNiff JM, Platt J, Bermudes D, Pawelek J. Melanoma x macrophage hybrids with enhanced metastatic potential. *Clin Exp Metastasis* 1998; **16**: 299-312 [PMID: 9626809 DOI: 10.1023/a:1006557228604]

73 **Rappa G**, Mercapide J, Lorico A. Spontaneous formation of tumorigenic hybrids between breast cancer and multipotent stromal cells is a source of tumor heterogeneity. *Am J Pathol* 2012; **180**: 2504-2515

[PMID: 22542847 DOI: 10.1016/j.ajpath.2012.02.020]
74 **Shen Z**, Gu X, Mao W, Cao H, Zhang R, Zhou Y, Liu K, Wang L, Zhang Z, Yin L. Dendritic cells fused with endothelial progenitor cells play immunosuppressive effects on angiogenesis in acute myeloid leukemia mice. *Am J Transl Res* 2019; **11**: 2816-2829 [PMID: 31217856]
75 **Busund LT**, Killie MK, Bartnes K, Seljelid R. Spontaneously formed tumorigenic hybrids of Meth A sarcoma and macrophages grow faster and are better vascularized than the parental tumor. *Int J Cancer* 2002; **100**: 407-413 [PMID: 12115521 DOI: 10.1002/ijc.10502]
76 **Kerjaschki D**, Huttary N, Raab I, Regele H, Bojarski-Nagy K, Bartel G, Kröber SM, Greinix H, Rosenmaier A, Karlhofer F, Wick N, Mazal PR. Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants. *Nat Med* 2006; **12**: 230-234 [PMID: 16415878 DOI: 10.1038/nm1340]
77 **Kerjaschki D**. The crucial role of macrophages in lymphangiogenesis. *J Clin Invest* 2005; **115**: 2316-2319 [PMID: 16138185 DOI: 10.1172/JCI26354]
78 **Kurahara H**, Takao S, Maemura K, Mataki Y, Kuwahata T, Maeda K, Sakoda M, Iino S, Ishigami S, Ueno S, Shinchi H, Natsugoe S. M2-polarized tumor-associated macrophage infiltration of regional lymph nodes is associated with nodal lymphangiogenesis and occult nodal involvement in pN0 pancreatic cancer. *Pancreas* 2013; **42**: 155-159 [PMID: 22699204 DOI: 10.1097/MPA.0b013e318254f2d1]
79 **Mantovani A**, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002; **23**: 549-555 [PMID: 12401408 DOI: 10.1016/s1471-4906(02)02302-5]
80 **Condeelis J**, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 2006; **124**: 263-266 [PMID: 16439202 DOI: 10.1016/j.cell.2006.01.007]
81 **Lin EY**, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN, Pollard JW. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006; **66**: 11238-11246 [PMID: 17114237 DOI: 10.1158/0008-5472.CAN-06-1278]
82 **Lin EY**, Pollard JW. Tumor-associated macrophages press the angiogenic switch in breast cancer. *Cancer Res* 2007; **67**: 5064-5066 [PMID: 17545580 DOI: 10.1158/0008-5472.CAN-07-0912]
83 **Pollard JW**. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004; **4**: 71-78 [PMID: 14708027 DOI: 10.1038/nrc1256]
84 **Cecconi O**, Calorini L, Mannini A, Mugnai G, Ruggieri S. Enhancement of lung-colonizing potential of murine tumor cell lines co-cultivated with activated macrophages. *Clin Exp Metastasis* 1997; **15**: 94-101 [PMID: 9062385 DOI: 10.1023/a:1018440508189]
85 **Oosterling SJ**, van der Bij GJ, Meijer GA, Tuk CW, van Garderen E, van Rooijen N, Meijer S, van der Sijp JR, Beelen RH, van Egmond M. Macrophages direct tumour histology and clinical outcome in a colon cancer model. *J Pathol* 2005; **207**: 147-155 [PMID: 16104052 DOI: 10.1002/path.1830]
86 **Miselis NR**, Wu ZJ, Van Rooijen N, Kane AB. Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma. *Mol Cancer Ther* 2008; **7**: 788-799 [PMID: 18375821 DOI: 10.1158/1535-7163.MCT-07-0579]
87 **Bingle L**, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* 2002; **196**: 254-265 [PMID: 11857487 DOI: 10.1002/path.1027]
88 **Tan SY**, Fan Y, Luo HS, Shen ZX, Guo Y, Zhao LJ. Prognostic significance of cell infiltrations of immunosurveillance in colorectal cancer. *World J Gastroenterol* 2005; **11**: 1210-1214 [PMID: 15754407 DOI: 10.3748/wjg.v11.i8.1210]
89 **Lackner C**, Jukic Z, Tsybrovskyy O, Jatzko G, Wette V, Hoefler G, Klimpfinger M, Denk H, Zatloukal K. Prognostic relevance of tumour-associated macrophages and von Willebrand factor-positive microvessels in colorectal cancer. *Virchows Arch* 2004; **445**: 160-167 [PMID: 15232739 DOI: 10.1007/s00428-004-1051-z]
90 **Sundar SS**, Ganesan TS. Role of lymphangiogenesis in cancer. *J Clin Oncol* 2007; **25**: 4298-4307 [PMID: 17878481 DOI: 10.1200/JCO.2006.07.1092]
91 **Johansson CB**, Youssef S, Koleckar K, Holbrook C, Doyonnas R, Corbel SY, Steinman L, Rossi FM, Blau HM. Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. *Nat Cell Biol* 2008; **10**: 575-583 [PMID: 18425116 DOI: 10.1038/ncb1720]
92 **Tavaluc RT**, Hart LS, Dicker DT, El-Deiry WS. Effects of low confluency, serum starvation and hypoxia on the side population of cancer cell lines. *Cell Cycle* 2007; **6**: 2554-2562 [PMID: 17912032 DOI: 10.4161/cc.6.20.4911]
93 **Mohyeldin A**, Garzón-Muvdi T, Quiñones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* 2010; **7**: 150-161 [PMID: 20682444 DOI: 10.1016/j.stem.2010.07.007]
94 **Noubissi FK**, Harkness T, Alexander CM, Ogle BM. Apoptosis-induced cancer cell fusion: a mechanism of breast cancer metastasis. *FASEB J* 2015; **29**: 4036-4045 [PMID: 26085132 DOI: 10.1096/fj.15-271098]
95 **Melzer C**, von der Ohe J, Hass R. In Vitro Fusion of Normal and Neoplastic Breast Epithelial Cells with Human Mesenchymal Stroma/Stem Cells Partially Involves Tumor Necrosis Factor Receptor Signaling. *Stem Cells* 2018; **36**: 977-989 [PMID: 29569804 DOI: 10.1002/stem.2819]
96 **Lopez-Sánchez LM**, Jimenez C, Valverde A, Hernandez V, Peñarando J, Martinez A, Lopez-Pedrera C, Muñoz-Castañeda JR, De la Haba-Rodríguez JR, Aranda E, Rodriguez-Ariza A. CoCl2, a mimic of hypoxia, induces formation of polyploid giant cells with stem characteristics in colon cancer. *PLoS One* 2014; **9**: e99143 [PMID: 24932611 DOI: 10.1371/journal.pone.0099143]
97 **Zhang Y**, Ilaslan H, Bauer TW. Giant cell tumor of bone: imaging and histology changes after denosumab treatment: Comment on: von Borstel D, Taguibao RA, Strle NA, Burns JE. Giant cell tumor of the bone: Aggressive case initially treated with denosumab and intralesional surgery. *Skeletal Radiol* 2017; **46**: 961-962 [PMID: 28389819 DOI: 10.1007/s00256-017-2643-4]
98 **Gentric G**, Desdouets C. Polyploidization in liver tissue. *Am J Pathol* 2014; **184**: 322-331 [PMID: 24140012 DOI: 10.1016/j.ajpath.2013.06.035]
99 **Qu Y**, Zhang L, Rong Z, He T, Zhang S. Number of glioma polyploid giant cancer cells (PGCCs) associated with vasculogenic mimicry formation and tumor grade in human glioma. *J Exp Clin Cancer Res* 2013; **32**: 75 [PMID: 24422894 DOI: 10.1186/1756-9966-32-75]
100 **Lewis JS**, Scantlebury JB, Luo J, Thorstad WL. Tumor cell anaplasia and multinucleation are predictors of disease recurrence in oropharyngeal squamous cell carcinoma, including among just the human papillomavirus-related cancers. *Am J Surg Pathol* 2012; **36**: 1036-1046 [PMID: 22743286 DOI: 10.1097/PAS.0b013e3182583678]

101 **Deangelis CE**, Zarrow E, Sochan O. Giant cell carcinoma of the lung. Report of a case. *Am Pract Dig Treat* 1961; **12**: 663-666 [PMID: 13720969]
102 **Nash AD**, Stout AP. Giant cell carcinoma of the lung; report of 5 cases. *Cancer* 1958; **11**: 369-376 [PMID: 13511359 DOI: 10.1002/1097-0142(195803/04)11:2<369::aid-cncr2820110222>3.0.co;2-8]
103 **Ozzello L**, Stout AP. The epithelial origin of giant cell carcinoma of the lung confirmed by tissue culture. Report of a case. *Cancer* 1961; **14**: 1052-1056 [PMID: 13731858 DOI: 10.1002/1097-0142(196109/10)14:5<1052::aid-cncr2820140521>3.0.co;2-d]
104 **Leske H**, Brandal P, Rushing EJ, Niehusmann P. IDH-mutant giant cell glioblastoma: A neglected tumor variant? *Clin Neuropathol* 2017; **36**: 293-295 [PMID: 29035191 DOI: 10.5414/NP301054]
105 **Weng SS**, Cao Y, Tang XJ, Zhu LZ, Tan YN, Dong CX, Chen JQ, Shen H, Yuan Y. Epidemiological features of lung giant cell carcinoma and therapy for patients with EGFR mutations based on case reports and the surveillance, epidemiology, and end results (SEER) database. *Oncotarget* 2017; **8**: 25323-25333 [PMID: 28445978 DOI: 10.18632/oncotarget.15831]
106 **Branca G**, Barresi V, Ieni A, Irato E, Caruso RA. Pleomorphic Carcinoma of the Colon: Morphological and Immunohistochemical Findings. *Case Rep Gastroenterol* 2016; **10**: 233-240 [PMID: 27462191 DOI: 10.1159/000446577]
107 **Adams DL**, Martin SS, Alpaugh RK, Charpentier M, Tsai S, Bergan RC, Ogden IM, Catalona W, Chumsri S, Tang CM, Cristofanilli M. Circulating giant macrophages as a potential biomarker of solid tumors. *Proc Natl Acad Sci USA* 2014; **111**: 3514-3519 [PMID: 24550495 DOI: 10.1073/pnas.1320198111]
108 **Clawson GA**, Matters GL, Xin P, Imamura-Kawasawa Y, Du Z, Thiboutot DM, Helm KF, Neves RI, Abraham T. Macrophage-tumor cell fusions from peripheral blood of melanoma patients. *PLoS One* 2015; **10**: e0134320 [PMID: 26267609 DOI: 10.1371/journal.pone.0134320]
109 **Clawson GA**, Matters GL, Xin P, McGovern C, Wafula E, dePamphilis C, Meckley M, Wong J, Stewart L, D'Jamoos C, Altman N, Imamura Kawasawa Y, Du Z, Honaas L, Abraham T. "Stealth dissemination" of macrophage-tumor cell fusions cultured from blood of patients with pancreatic ductal adenocarcinoma. *PLoS One* 2017; **12**: e0184451 [PMID: 28957348 DOI: 10.1371/journal.pone.0184451]
110 **Illidge TM**, Cragg Cragg MS, Fringes B, Olive P, Erenpreisa JA. Polyploid giant cells provide a survival mechanism for p53 mutant cells after DNA damage. *Cell Biol Int* 2000; **24(9)**: 621-633 [PMID: 10964452 DOI: 10.1006/cbir.2000.0557]
111 **Schoonen PM**, Talens F, Stok C, Gogola E, Heijink AM, Bouwman P, Foijer F, Tarsounas M, Blatter S, Jonkers J, Rottenberg S, van Vugt MATM. Progression through mitosis promotes PARP inhibitor-induced cytotoxicity in homologous recombination-deficient cancer cells. *Nat Commun* 2017; **8**: 15981 [PMID: 28714471 DOI: 10.1038/ncomms15981]
112 **Erenpreisa J**, Cragg MS, Anisimov AP, Illidge TM. Tumor cell embryonality and the ploidy number 32n: is it a developmental checkpoint? *Cell Cycle* 2011; **10**: 1873-1874 [PMID: 21519193 DOI: 10.4161/cc.10.11.15755]
113 **Ivanov A**, Cragg MS, Erenpreisa J, Emzinsh D, Lukman H, Illidge TM. Endopolyploid cells produced after severe genotoxic damage have the potential to repair DNA double strand breaks. *J Cell Sci***116** (Pt 20): 4095-4106 [PMID: 12953071 DOI: 10.1242/jcs.00740]
114 **Erenpreisa JA**, Cragg MS, Fringes B, Sharakhov I, Illidge TM. Release of mitotic descendants by giant cells from irradiated Burkitt's lymphoma cell line. *Cell Biol Int* 2000; **24(9)**: 635-648 [PMID: 10964453 DOI: 10.1006/cbir.2000.0558]
115 **Carloni V**, Mazzocca A, Mello T, Galli A, Capaccioli S. Cell fusion promotes chemoresistance in metastatic colon carcinoma. *Oncogene* 2013; **32**: 2649-2660 [PMID: 22751128 DOI: 10.1038/onc.2012.268]
116 **Puig PE**, Guilly MN, Bouchot A, Droin N, Cathelin D, Bouyer F, Favier L, Ghiringhelli F, Kroemer G, Solary E, Martin F, Chauffert B. Tumor cells can escape DNA-damaging cisplatin through DNA endoreduplication and reversible polyploidy. *Cell Biol Int* 2008; **32**: 1031-1043 [PMID: 18550395 DOI: 10.1016/j.cellbi.2008.04.021]
117 **Weihua Z**, Lin Q, Ramoth AJ, Fan D, Fidler IJ. Formation of solid tumors by a single multinucleated cancer cell. *Cancer* 2011; **117**: 4092-4099 [PMID: 21365635 DOI: 10.1002/cncr.26021]
118 **Silk AD**, Gast CE, Davies PS, Fakhari FD, Vanderbeek GE, Mori M, Wong MH. Fusion between hematopoietic and epithelial cells in adult human intestine. *PLoS One* 2013; **8**: e55572 [PMID: 23383228 DOI: 10.1371/journal.pone.0055572]
119 **Ambarus CA**, Krausz S, van Eijk M, Hamann J, Radstake TR, Reedquist KA, Tak PP, Baeten DL. Systematic validation of specific phenotypic markers for in vitro polarized human macrophages. *J Immunol Methods* 2012; **375**: 196-206 [PMID: 22075274 DOI: 10.1016/j.jim.2011.10.013]
120 **Ma C**, Horlad H, Ohnishi K, Nakagawa T, Yamada S, Kitada S, Motoshima T, Kamba T, Nakayama T, Fujimoto N, Takeya M, Komohara Y. CD163-positive cancer cells are potentially associated with high malignant potential in clear cell renal cell carcinoma. *Med Mol Morphol* 2018; **51**: 13-20 [PMID: 28687956 DOI: 10.1007/s00795-017-0165-8]
121 **Shabo I**, Stål O, Olsson H, Doré S, Svanvik J. Breast cancer expression of CD163, a macrophage scavenger receptor, is related to early distant recurrence and reduced patient survival. *Int J Cancer* 2008; **123**: 780-786 [PMID: 18506688 DOI: 10.1002/ijc.23527]
122 **Shabo I**, Olsson H, Elkarim R, Sun XF, Svanvik J. Macrophage Infiltration in Tumor Stroma is Related to Tumor Cell Expression of CD163 in Colorectal Cancer. *Cancer Microenviron* 2014; **7**: 61-69 [PMID: 24771466 DOI: 10.1007/s12307-014-0145-7]
123 **Shabo I**, Olsson H, Sun XF, Svanvik J. Expression of the macrophage antigen CD163 in rectal cancer cells is associated with early local recurrence and reduced survival time. *Int J Cancer* 2009; **125**: 1826-1831 [PMID: 19582880 DOI: 10.1002/ijc.24506]
124 **Aljabery F**, Olsson H, Gimm O, Jahnson S, Shabo I. M2-macrophage infiltration and macrophage traits of tumor cells in urinary bladder cancer. *Urol Oncol* 2018; **36**: 159.e19-159.e26 [PMID: 29288002 DOI: 10.1016/j.urolonc.2017.11.020]
125 **Maniecki MB**, Etzerodt A, Ulhøi BP, Steiniche T, Borre M, Dyrskjøt L, Orntoft TF, Moestrup SK, Møller HJ. Tumor-promoting macrophages induce the expression of the macrophage-specific receptor CD163 in malignant cells. *Int J Cancer* 2012; **131**: 2320-2331 [PMID: 22362417 DOI: 10.1002/ijc.27506]
126 **Shabo I**, Midtbö K, Andersson H, Åkerlund E, Olsson H, Wegman P, Gunnarsson C, Lindström A. Macrophage traits in cancer cells are induced by macrophage-cancer cell fusion and cannot be explained by cellular interaction. *BMC Cancer* 2015; **15**: 922 [PMID: 26585897 DOI: 10.1186/s12885-015-1935-0]
127 **Lindström A**, Midtbö K, Arnesson LG, Garvin S, Shabo I. Fusion between M2-macrophages and cancer

128 **Davies PS**, Powell AE, Swain JR, Wong MH. Inflammation and proliferation act together to mediate intestinal cell fusion. *PLoS One* 2009; **4**: e6530 [PMID: 19657387 DOI: 10.1371/journal.pone.0006530]

129 **Miller FR**, McInerney D, Rogers C, Miller BE. Spontaneous fusion between metastatic mammary tumor subpopulations. *J Cell Biochem* 1988; **36**: 129-136 [PMID: 3356752 DOI: 10.1002/jcb.240360204]

130 **Del Monte U**. Does the cell number 10(9) still really fit one gram of tumor tissue? *Cell Cycle* 2009; **8**: 505-506 [PMID: 19176997 DOI: 10.4161/cc.8.3.7608]

131 **Garvin S**, Vikhe Patil E, Arnesson LG, Oda H, Hedayati E, Lindström A, Shabo I. Differences in intra-tumoral macrophage infiltration and radiotherapy response among intrinsic subtypes in pT1-T2 breast cancers treated with breast-conserving surgery. *Virchows Arch* 2019; **475**: 151-162 [PMID: 30915533 DOI: 10.1007/s00428-019-02563-3]

132 **Lee HR**, Hwang KA, Park MA, Yi BR, Jeung EB, Choi KC. Treatment with bisphenol A and methoxychlor results in the growth of human breast cancer cells and alteration of the expression of cell cycle-related genes, cyclin D1 and p21, via an estrogen receptor-dependent signaling pathway. *Int J Mol Med* 2012; **29**: 883-890 [PMID: 22307313 DOI: 10.3892/ijmm.2012.903]

133 **Ding J**, Jin W, Chen C, Shao Z, Wu J. Tumor associated macrophage × cancer cell hybrids may acquire cancer stem cell properties in breast cancer. *PLoS One* 2012; **7**: e41942 [PMID: 22848668 DOI: 10.1371/journal.pone.0041942]

134 **Rupani R**, Handerson T, Pawelek J. Co-localization of beta1,6-branched oligosaccharides and coarse melanin in macrophage-melanoma fusion hybrids and human melanoma cells in vitro. *Pigment Cell Res* 2004; **17**: 281-288 [PMID: 15140074 DOI: 10.1111/j.1600-0749.2004.00148.x]

135 **Shabo I**, Olsson H, Stål O, Svanvik J. Breast cancer expression of DAP12 is associated with skeletal and liver metastases and poor survival. *Clin Breast Cancer* 2013; **13**: 371-377 [PMID: 23810293 DOI: 10.1016/j.clbc.2013.05.003]

136 **Nguyen TT**, Schwartz EJ, West RB, Warnke RA, Arber DA, Natkunam Y. Expression of CD163 (hemoglobin scavenger receptor) in normal tissues, lymphomas, carcinomas, and sarcomas is largely restricted to the monocyte/macrophage lineage. *Am J Surg Pathol* 2005; **29**: 617-624 [PMID: 15832085 DOI: 10.1097/01.pas.0000157940.80538.ec]

137 **Friedl P**. Cell fusion: new mechanisms of plasticity in cancer? *Lancet Oncol* 2005; **6**: 916-918 [PMID: 16321755 DOI: 10.1016/S1470-2045(05)70439-3]

138 **Dittmar T**, Schwitalla S, Seidel J, Haverkampf S, Reith G, Meyer-Staeckling S, Brandt BH, Niggemann B, Zänker KS. Characterization of hybrid cells derived from spontaneous fusion events between breast epithelial cells exhibiting stem-like characteristics and breast cancer cells. *Clin Exp Metastasis* 2011; **28**: 75-90 [PMID: 20981475 DOI: 10.1007/s10585-010-9359-3]

139 **Gal H**, Krizhanovsky V. Cell fusion induced senescence. *Aging (Albany NY)* 2014; **6**: 353-354 [PMID: 24859630 DOI: 10.18632/aging.100670]

140 **Mohr M**, Zaenker KS, Dittmar T. Fusion in cancer: an explanatory model for aneuploidy, metastasis formation, and drug resistance. *Methods Mol Biol* 2015; **1313**: 21-40 [PMID: 25947654 DOI: 10.1007/978-1-4939-2703-6_2]

141 **Krug S**, Abbassi R, Griesmann H, Sipos B, Wiese D, Rexin P, Blank A, Perren A, Haybaeck J, Hüttelmaier S, Rinke A, Gress TM, Michl P. Therapeutic targeting of tumor-associated macrophages in pancreatic neuroendocrine tumors. *Int J Cancer* 2018; **143**: 1806-1816 [PMID: 29696624 DOI: 10.1002/ijc.31562]

142 **Nakatsumi H**, Matsumoto M, Nakayama KI. Noncanonical Pathway for Regulation of CCL2 Expression by an mTORC1-FOXK1 Axis Promotes Recruitment of Tumor-Associated Macrophages. *Cell Rep* 2017; **21**: 2471-2486 [PMID: 29186685 DOI: 10.1016/j.celrep.2017.11.014]

143 **Guth AM**, Hafeman SD, Elmslie RE, Dow SW. Liposomal clodronate treatment for tumour macrophage depletion in dogs with soft-tissue sarcoma. *Vet Comp Oncol* 2013; **11**: 296-305 [PMID: 22540967 DOI: 10.1111/j.1476-5829.2012.00319.x]

144 **Reusser NM**, Dalton HJ, Pradeep S, Gonzalez-Villasana V, Jennings NB, Vasquez HG, Wen Y, Rupaimoole R, Nagaraja AS, Gharpure K, Miyake T, Huang J, Hu W, Lopez-Berestein G, Sood AK. Clodronate inhibits tumor angiogenesis in mouse models of ovarian cancer. *Cancer Biol Ther* 2014; **15**: 1061-1067 [PMID: 24841852 DOI: 10.4161/cbt.29184]

145 **Griesmann H**, Drexel C, Milosevic N, Sipos B, Rosendahl J, Gress TM, Michl P. Pharmacological macrophage inhibition decreases metastasis formation in a genetic model of pancreatic cancer. *Gut* 2017; **66**: 1278-1285 [PMID: 27013602 DOI: 10.1136/gutjnl-2015-310049]

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ORIGINAL ARTICLE

Retrospective Study

What factors influence patient experience in orthopedic oncology office visits?

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Abstract

BACKGROUND

Patient satisfaction and reported outcomes are becoming increasingly important in determining the efficacy of clinical care. To date no study has evaluated the patient experience in the orthopedic oncology outpatient setting to determine which factors of the encounter are priorities to the patient.

AIM

To evaluate what factors impact patient experience and report satisfaction in an outpatient orthopedic oncology clinic.

METHODS

Press Ganey® patient surveys from a single outpatient orthopedic oncology clinic at a tertiary care setting were prospectively collected per routine medical care. All orthopedic oncology patients who were seen in clinic and received electronic survey were included. All survey responses were submitted within one month of clinic appointment. IRB approval was obtained to retrospectively collect survey responses from 2015 to 2016. Basic demographic data along with survey category responses were collected and statistically analyzed.

RESULTS

One hundred sixty-two patient surveys were collected. Average patient age was

Data sharing statement: No additional data are available.

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54.4 years (SD = 16.2 years) and were comprised of 51.2% female and 48.4% male. 64.2% of patients were from in-state. Out of state residents were more likely to recommend both the practice and attending physician. The likelihood to recommend attending physician was positively associated with MD friendliness/courtesy (OR = 14.4, 95%CI: 2.5-84.3), MD confidence (OR = 48.2, 95%CI: 6.2-376.5), MD instructions follow-up care (OR = 2.5, 95%CI: 0.4-17.4), and sensitivity to needs (OR = 16.1, 95%CI: 1-262.5). Clinic operations performed well in the categories of courtesy of staff (76%) and cleanliness (75%) and less well in ease of getting on the phone (49%), information about delays (36%), and wait time (37%).

CONCLUSION
Orthopedic specialties can utilize information from this study to improve care from the patient perspective. Future studies may be directed at how to improve these areas of care which are most valued by the patient.

Key words: Press Ganey® survey; Orthopedic oncology; Outpatient clinic; Patient experience; Patient satisfaction; Patient reported outcomes

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Core tip: Patient satisfaction and reported outcomes play a vital role in determining the efficacy of clinical care in both inpatient and outpatient settings. This study addressed factors of the outpatient orthopedic oncology clinic that were found to be important to the patient. Provider friendliness, confidence, and sensitivity to needs, as perceived from the patient, were all associated with increased likelihood of the patient to recommend the attending physician to others. The findings from this study can guide various outpatient oncology clinics on how to research and improve patient satisfaction and reported outcomes.

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INTRODUCTION

Patient satisfaction and patient reported outcomes are becoming increasingly important in determining the efficacy of clinical care. For decades in the orthopedic oncology literature, overall survival, metastasis and local recurrence statistics have been well published[1-4]. Using these metrics, patients were determined to be doing well if they were alive, free of local or metastatic disease at various time points. Assessment of patient functional outcomes has been evaluated using scoring systems such as the musculoskeletal tumor society score and Toronto extremity salvage score. However, not until more recently have physicians begun to evaluate various functional, pain related, and psychological outcomes[5-8]. Since this recent interest, the literature has shown that using these metrics can provide valuable information regarding how patients are functioning on a physical and psychological level while being treated as well as during surveillance.

Overall patient satisfaction scores and patient reported outcomes are challenging measurements to understand[9-11]. Each patient has a unique personality as well as pathology and these can both affect patient satisfaction scores. There has been some conflicting data published studying the subject of how patient satisfaction correlates to efficacy of clinical care. Some studies have found positive correlations to efficacy of care and others have found the opposite[9,12]. Understanding the patient perspective certainly provides the care team insight regarding the patients' perceived strengths and weaknesses of care. Furthermore, patient satisfaction scores may in part determine the future of hospital and physician reimbursements[13]. Press Ganey® medical practice outpatient and ambulatory surgery patient experience of care survey ("Press Ganey survey") is a questionnaire used to evaluate patient satisfaction in the

outpatient clinic setting. The number of cancer patients and cancer related costs in the United States is significant and rises every year, hence we feel this is an important demographic to further investigate[14]. In this study, our group evaluated prospectively collected survey information to determine what factors contributed to patients' likelihood to recommend our practice as well as our providers in our orthopedic oncology outpatient clinic. The study also aimed to identify areas of our clinical practice that were rated highly and those that needed improvement from the patient perspective.

Patient satisfaction surveys have the capacity to provide the orthopedic care team with a window into the perspective of the patient. Although it is unclear whether patient satisfaction scores are directly or indirectly related to efficacy of care this information provides insight to the care team and can help to implement reasonable and necessary changes. The Press Ganey survey is a questionnaire used in the outpatient setting to gather this information from the patient perspective. No study to date has evaluated which factors within the Press Ganey survey are most valued by orthopedic oncology patients. Further, no study to date has reviewed Press Ganey survey results from an orthopedic oncology practice outpatient setting in order to determine practice strengths and weaknesses. We feel the results of this study can provide valuable information about the patient perspective in a clinic setting which can be useful for not just orthopedic oncology clinics but all orthopedic outpatient clinics.

MATERIALS AND METHODS

Patients and methods

We performed an IRB approved retrospective review of prospectively collected Press Ganey survey data from our orthopedic oncology outpatient clinic. The Press Ganey survey was distributed electronically to all patients seen in the outpatient orthopedic oncology clinic. The Press Ganey survey consists of 37 questions involving the clinical check-in process, facility quality, ease of communication with clinic staff, surgical educational information, and overall patient experience. The full survey takes approximately 15 min to complete. Computer adaptive versions are available which shorten the survey to 5-10 questions and allow for completion in as little as 5 min. Patients have 30 d from their clinic encounter to complete the questionnaire. Data was collected during the 2015 and 2016 calendar year. One hundred sixty-two completed the Press Ganey surveys from the outpatient orthopedic oncology clinic and responses were prospectively collected in order to retrospectively review. Basic demographic data along with survey category responses were collected.

Statistical analysis

Data was recorded electronically and obtained in accordance with the medical center's IRB protocol. The data was then submitted to the University Study Design and Biostatistics Center for statistical evaluation. Frequency and percent were presented for all categorical variables as well as for outcome variables "likelihood to recommend the physician" and "likelihood to recommend the practice". Analysis of variance was applied continuous variables and χ^2-test/Fisher's exact test was applied to assess between categorical variables. All bivariate analyses were two-tailed and the statistical significance was set as $P < 0.05$. Firth logistic regression analyses were performed using least absolute shrinkage and selection operator (LASSO) to identify predictive factors for each outcome of interest.

Due to the low frequencies of some answers in the survey all original variables with results-0, 25, 50, 75, 100 and N/A were re-categorized as not completely satisfied (0/25/50/75), completely satisfied (100) and N/A.

Descriptive and bivariate analyses were based on the analysis data set composed by every patient's first record. Mean, standard deviation, median, minimum and maximum were presented for numeric variables. Frequency and percent were presented for categorical variables in overall, and by outcome variables "likelihood to recommend the physician" and "likelihood to recommend the practice". Analysis of variance was applied to assess the possible difference in mean of continuous variable with approximately normal distribution among groups specified by outcome variable. χ^2-test or Fisher's exact test was applied to assess the possible association between two categorical variables. All bivariate analyses were two-tailed and the statistical significance was set as $P < 0.05$.

Based on results from previous steps and nature of the data (*e.g.*, rare events and quasi-complete separation), Firth logistic regression analyses were performed using LASSO to identify predictive factors truly informative for each outcome of interest,

and the final model for each outcome was determined based on the optimal tuning parameter adopting 10 fold cross-validation criteria. The final models were unpenalized; odds ratios and 95% confidence intervals were reported.

Statistical software was SAS 9.4 (SAS Institute Inc., Cary, NC, United States) and R (www.r-project.org). All LASSO analyses were conducted using the "glmnet" package in R. All statistical methods were reviewed by Yue Zhang and Wei J Liu from the University of Utah.

RESULTS

Among the 162 participants, regarding patient's characteristics, the average age was 54.4 years (standard deviation = 16.2 years, median = 56 years, range = 17 to 95 years); 83 (51.2%) participants were female; 104 (64.2%) participants were from in-state; 89 (54.9%) participants had malignant diagnosis.

Regarding the outcomes "likelihood to recommend the physician/likelihood to recommend the practice" and patient's characteristics, it was statistically significant on bivariate analysis that participants who lived out of state were more likely to have complete satisfaction of "likelihood to recommend the physician/likelihood to recommend the practice" compared to participants who lived in state (91.4% *vs* 73.1%, $P = 0.027$; 86.2% *vs* 69.2%, $P = 0.029$, χ^2-test); among other patient characteristics, there were no statistically significant relationships observed ($P > 0.02$, $P > 0.05$ in all instances, ANOVA, χ^2-test or Fisher's exact test).

Regarding outcomes "likelihood to recommend the physician/likelihood to recommend the practice" and individual question responses, it was statistically significant on bivariate analysis that, for each item participants reported "Completely satisfied" they were more likely to have complete satisfaction of "likelihood to recommend the physician" compared to participants who reported "Not completely satisfied" ($P < 0.004$, $P < 0.001$ in all instances, χ^2-test or Fisher's exact test). The outcome "likelihood to recommend the physician/likelihood to recommend the practice" was statistically associated with each individual survey question answered on bivariate analysis ($P < 0.01$, $P < 0.001$ in all instances, Fisher's exact test).

Regarding the model of "likelihood to recommend the physician", Firth logistic regression analysis (with LASSO regulation) results suggested that complete satisfaction of "likelihood to recommend the physician" was positively associated with complete satisfaction of factors including MD friendliness/courtesy (OR = 14.4, 95%CI: 2.5-84.3), MD confidence (OR = 48.2, 95%CI: 6.2-376.5), MD instructions follow up care (OR = 2.5, 95%CI: 0.4-7.4), sensitivity to needs (OR = 16.1 , 95%CI: 1-62.5) (Table 1).

Regarding model of "likelihood to recommend the practice", Firth logistic regression analysis (with LASSO regulation) results suggested that complete satisfaction of "likelihood to recommend the practice" was positively associated with complete satisfaction of factors including MD confidence (OR = 11.6, 95%CI: 2.1-63.4), sensitivity to needs (OR = 5.8, 95%CI: 1.3-26.7), staff work together (OR = 36.1, 95%CI: 7.9-165.1) (Table 1).

DISCUSSION

Disease related outcomes have been the gold standard in outcomes measures when discussing oncology patients for many years. Indeed, knowing local recurrence, metastasis and overall survival rates are crucial in understanding the efficacy of treatments. However, functional outcomes and patient reported outcomes also have critical roles in better describing how patients are living after their diagnosis is made and treatment undertaken.

In the orthopedic oncology literature functional outcomes have adapted over time starting with general evaluations such as the SF-36. Functional outcomes then became somewhat more orthopedic based and included tests such as the Harris hip, Oxford knee and DASH scores. From that point, functional evaluations have been developed specifically for orthopedic oncology and include the musculoskeletal tumor society score and Toronto extremity salvage scoring system[1-3]. A relatively new development in patient reported outcomes is the utilization of Patient Reported Outcomes Measurement Information System, which is a simple, computer adaptive test that has been utilized in the oncology literature recently[15-17].

The next progression in evaluating care from the patient perspective comes in the form of patient satisfaction surveys. These surveys, such as Press Ganey, focus on the purely subjective aspect of which aspects of their care they found to be acceptable,

Table 1 Factors for likelihood to recommend physician and practice

Variable (completely *vs* not completely satisfied)	OR	95%CI
Likelihood to recommend the physician		
MD confidence	48.2	(6.2, 376.5)
Sensitivity to needs	16.6	(1, 262.5)
MD friendliness/courtesy	14.4	(2.5, 84.3)
MD instructions on follow-up	2.5	(0.4, 17.4)
Likelihood to recommend the practice		
Staff working together	36.1	(7.9, 165.1)
MD confidence	11.6	(2.1, 63.4)
Sensitivity to needs	5.8	(1.3, 26.7)

and which needed improvement[8,18-20]. There is some debate in the literature as to whether patient satisfaction is positively or negatively correlated with efficacy of care[21,22]. Fenton *et al*[9] in 2012 published results of a large prospective series and found that higher patient satisfaction was associated with more admissions, more prescription drugs, more overall expenditures and a higher mortality rate. Chang *et al*[7] in 2006 published their results that among a geriatric cohort of 236 patients, assessments of quality of care was not related to global ratings of care. The group recommended against using global ratings of care to be used as a marker of technical quality of care[7]. In the orthopedic literature, Chughtai *et al*[18] published their results of a retrospective study involving 736 knee replacement patients and found there was no statistically significant relationship between Press Ganey survey scores and standard arthroplasty outcomes measures.

Conversely Tsai *et al*[12] published in 2015 that among a large cohort of surgical patients, hospitals with higher patient satisfaction provided more efficient care with fewer readmissions and lower mortality rates. Similarly, Sacks *et al*[23] in 2015 published results of a retrospective study of over 100,000 patients and found that patients treated in hospitals which ranked in the highest satisfaction quartile had significantly lower risk of death and minor complications compared to those in the lowest satisfaction quartile.

Regardless of the correlation, important information can be gathered from the survey data collected in this study. In the oncology patient population, we believe that elucidating which factors of the clinic experience are perceived positively and which negatively can help the care team to improve the overall patient experience while not compromising clinical care.

Our study certainly has several limitations including the response rate of 17% overall. Other limitations include the ability of patients to complete surveys up to 30 d after the clinical encounter which may affect patient recall ability. Our clinical practice is quite diverse, with some patients presenting for general orthopedic complaints and a distant history of cancer, and some currently undergoing treatment for sarcoma. We believe that this is in fact the typical patient population for an orthopedic oncology clinic and the heterogeneity increases our external validity. Finally, there is no consensus as to whether patient satisfaction scores are positively or negatively correlated with clinical outcomes. Because of this it is quite difficult to determine if implementing changes based on the outcomes of the satisfaction scores is appropriate in all settings.

The results of this study demonstrate that out-of-state patients may be more likely to be satisfied with their care than inpatients. The group had a difficult time explaining this finding but believes may be related to the fact that our clinical practice has a very wide catchment area and often patients will travel more than 500 miles for care. Those traveling very far are from more rural areas and often have a difficult time finding appropriate physicians for treatment in their hometown, which contributes to their unique perspective.

The data demonstrated that patients perceived our clinic to perform well in the categories of confidence in MD (81%), MD concern about worries/questions (75%), courtesy of staff (76%), MD friendliness/courtesy (79%), cleanliness (75%), MD including you in decision making (72%) and MD explained condition (75%). The clinic showed room for improvement in the categories of ease of getting on the phone (49%), information about delays (36%), wait time (37%) and MD speaking about medications (45%). Our outpatient clinic found this information useful in regards to areas which were satisfactory and which needed improvement and simple measures will be taken

to address those insufficiencies while not compromising clinical care.

The data demonstrated that the patients' likelihood to recommend the practice was positively associated with confidence in the physician, sensitivity to their needs and the staff working together. The data also demonstrated that the patients' likelihood to recommend the physician was positively associated with the courtesy of the physician, confidence in the physician, physician instructions on follow up care, and sensitivity to the patients' needs. These findings are also reasonable categories which patients would find most important and should be considered during every patient interaction.

We believe that by understanding which aspects of patient care are important to the patient, we can continue to improve the patient experience without compromising clinical care. The literature has shown that in some circumstance's patient reported outcomes may be negatively correlated to clinical outcomes while at other times they are positively associated[9,12]. In both situations, useful information can be obtained from better understanding the patient perspective. The outpatient clinic of study was able to determine which areas of their care were perceived as sufficient and which were insufficient and thus was able to make reasonable and appropriate changes without changing the overall care algorithm. This information may be very useful to orthopedic oncology clinics in addition to the various surgical oncology clinics in the quest to improve on patient experiences in a clinic setting.

ARTICLE HIGHLIGHTS

Research background

Patient satisfaction and reported outcomes play an important role in determining efficacy of clinical care. Little is known about the patient experience in an orthopedic oncology outpatient clinic.

Research motivation

This study aims to evaluate the potential factors that impact the patient experience within an outpatient orthopedic oncology clinic. Identification of these factors will allow us and others to improve the patient experience.

Research objectives

The primary objective of this study was to identify potentially modifiable factors that impact the patient reported experience. With this knowledge one can implement strategies to improve the outpatient experience.

Research methods

This study was a retrospective review of prospectively collected data obtained through routine medical care at a single orthopedic oncology outpatient clinic.

Research results

This study identified that most patients within the practice were from out of state. Likelihood to recommend the attending physician was associated with MD friendliness/courtesy, MD confidence, MD instructions follow-up care, and sensitivity to needs. Although the clinic operation performed well in the categories of courtesy of staff and cleanliness there is room for improvement in ease of getting on the phone, information about delays, and wait time.

Research conclusions

Orthopedic specialties can greatly benefit with the knowledge obtained from this study by understanding which factors are associated the patient experience in an outpatient clinic. Future studies can be aimed at improving areas of care identified from this study.

Research perspectives

The patient experience and reported satisfaction is becoming an important measure of clinical efficacy through various surgical and medical specialties. Little is known on the patient experience within an orthopedic oncology outpatient clinic. Future research is required to investigate strategies at improving areas within the outpatient clinic identified from this study.

REFERENCES

1 **Ahlmann ER**, Menendez LR, Kermani C, Gotha H. Survivorship and clinical outcome of modular endoprosthetic reconstruction for neoplastic disease of the lower limb. *J Bone Joint Surg Br* 2006; **88**: 790-795 [PMID: 16720775 DOI: 10.1302/0301-620X.88B6.17519]

2 **Gupta AA**, Pappo A, Saunders N, Hopyan S, Ferguson P, Wunder J, O'Sullivan B, Catton C, Greenberg M, Blackstein M. Clinical outcome of children and adults with localized Ewing sarcoma: impact of chemotherapy dose and timing of local therapy. *Cancer* 2010; **116**: 3189-3194 [PMID: 20564643 DOI: 10.1002/cncr.25144]

3 **Lee FY**, Mankin HJ, Fondren G, Gebhardt MC, Springfield DS, Rosenberg AE, Jennings LC. Chondrosarcoma of bone: an assessment of outcome. *J Bone Joint Surg Am* 1999; **81**: 326-338 [PMID: 10199270 DOI: 10.2106/00004623-199903000-00004]

4 **Shin KH**, Rougraff BT, Simon MA. Oncologic outcomes of primary bone sarcomas of the pelvis. *Clin Orthop Relat Res* 1994; **(304)**: 207-217 [PMID: 8020218 DOI: 10.1097/00003086-199407000-00033]

5 **Arvinius C**, Parra JL, Mateo LS, Maroto RG, Borrego AF, Stern LL. Benefits of early intramedullary nailing in femoral metastases. *Int Orthop* 2014; **38**: 129-132 [PMID: 24126497 DOI: 10.1007/s00264-013-2108-x]

6 **Bunnell CA**, Weingart SN, Swanson S, Mamon HJ, Shulman LN. Models of multidisciplinary cancer care: physician and patient perceptions in a comprehensive cancer center. *J Oncol Pract* 2010; **6**: 283-288 [PMID: 21358956 DOI: 10.1200/JOP.2010.000138]

7 **Chang JT**, Hays RD, Shekelle PG, MacLean CH, Solomon DH, Reuben DB, Roth CP, Kamberg CJ, Adams J, Young RT, Wenger NS. Patients' global ratings of their health care are not associated with the technical quality of their care. *Ann Intern Med* 2006; **144**: 665-672 [PMID: 16670136 DOI: 10.7326/0003-4819-144-9-200605020-00010]

8 **Dawson J**, Doll H, Fitzpatrick R, Jenkinson C, Carr AJ. The routine use of patient reported outcome measures in healthcare settings. *BMJ* 2010; **340**: c186 [PMID: 20083546 DOI: 10.1136/bmj.c186]

9 **Fenton JJ**, Jerant AF, Bertakis KD, Franks P. The cost of satisfaction: a national study of patient satisfaction, health care utilization, expenditures, and mortality. *Arch Intern Med* 2012; **172**: 405-411 [PMID: 22331982 DOI: 10.1001/archinternmed.2011.1662]

10 **Ponsford J**, Hill B, Karamitsios M, Bahar-Fuchs A. Factors influencing outcome after orthopedic trauma. *J Trauma* 2008; **64**: 1001-1009 [PMID: 18404068 DOI: 10.1097/TA.0b013e31809fec16]

11 **Wylde V**, Blom AW, Whitehouse SL, Taylor AH, Pattison GT, Bannister GC. Patient-reported outcomes after total hip and knee arthroplasty: comparison of midterm results. *J Arthroplasty* 2009; **24**: 210-216 [PMID: 18534427 DOI: 10.1016/j.arth.2007.12.001]

12 **Tsai TC**, Orav EJ, Jha AK. Patient satisfaction and quality of surgical care in US hospitals. *Ann Surg* 2015; **261**: 2-8 [PMID: 24887985 DOI: 10.1097/SLA.0000000000000765]

13 **Parr K**. Health literacy: improving understanding of discharge instructions. 2017. Available from: https://works.bepress.com/kimberly-parr/1/

14 **American Cancer Society**. Cancer Facts & Figures. 2005. Available from: http://www.cancer.org/acs/groups/content/@nho/documents/ document/caff2005f4pwsecuredpdf.pdf

15 **Hinds PS**, Nuss SL, Ruccione KS, Withycombe JS, Jacobs S, DeLuca H, Faulkner C, Liu Y, Cheng YI, Gross HE, Wang J, DeWalt DA. PROMIS pediatric measures in pediatric oncology: valid and clinically feasible indicators of patient-reported outcomes. *Pediatr Blood Cancer* 2013; **60**: 402-408 [PMID: 22829446 DOI: 10.1002/pbc.24233]

16 **McBride JS**, Anderson RT, Bahnson JL. Using a hand-held computer to collect data in an orthopedic outpatient clinic: a randomized trial of two survey methods. *Med Care* 1999; **37**: 647-651 [PMID: 10424635 DOI: 10.1097/00005650-199907000-00004]

17 **Wilke B**, Cooper A, Scarborough M, Gibbs CP, Spiguel A. An Evaluation of PROMIS Health Domains in Sarcoma Patients Compared to the United States Population. *Sarcoma* 2019; **2019**: 9725976 [PMID: 30799982 DOI: 10.1155/2019/9725976]

18 **Chughtai M**, Patel NK, Gwam CU, Khlopas A, Bonutti PM, Delanois RE, Mont MA. Do Press Ganey Scores Correlate With Total Knee Arthroplasty-Specific Outcome Questionnaires in Postsurgical Patients? *J Arthroplasty* 2017; **32**: S109-S112 [PMID: 28189440 DOI: 10.1016/j.arth.2017.01.007]

19 **Etier BE Jr**, Orr SP, Antonetti J, Thomas SB, Theiss SM. Factors impacting Press Ganey patient satisfaction scores in orthopedic surgery spine clinic. *Spine J* 2016; **16**: 1285-1289 [PMID: 27084192 DOI: 10.1016/j.spinee.2016.04.007]

20 **Presson AP**, Zhang C, Abtahi AM, Kean J, Hung M, Tyser AR. Psychometric properties of the Press Ganey® Outpatient Medical Practice Survey. *Health Qual Life Outcomes* 2017; **15**: 32 [PMID: 28183312 DOI: 10.1186/s12955-017-0610-3]

21 **Graham B**, Green A, James M, Katz J, Swiontkowski M. Measuring patient satisfaction in orthopaedic surgery. *J Bone Joint Surg Am* 2015; **97**: 80-84 [PMID: 25568398 DOI: 10.2106/JBJS.N.00811]

22 **Halm EA**, Lee C, Chassin MR. Is volume related to outcome in health care? A systematic review and methodologic critique of the literature. *Ann Intern Med* 2002; **137**: 511-520 [PMID: 12230353]

23 **Sacks GD**, Lawson EH, Dawes AJ, Russell MM, Maggard-Gibbons M, Zingmond DS, Ko CY. Relationship Between Hospital Performance on a Patient Satisfaction Survey and Surgical Quality. *JAMA Surg* 2015; **150**: 858-864 [PMID: 26108091 DOI: 10.1001/jamasurg.2015.1108]

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ORIGINAL ARTICLE

Retrospective Study

Efficacy, patterns of use and cost of Pertuzumab in the treatment of HER2+ metastatic breast cancer in Singapore: The National Cancer Centre Singapore experience

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Abstract

BACKGROUND

Pertuzumab is a humanized anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibody found in a Phase III clinical trial to significantly improve median survival in HER2 positive metastatic breast cancer (MBC) when used in combination with a taxane and Trastuzumab, and its clinical efficacy has transformed the therapeutic landscape of HER2-positive breast cancer. There are currently few reports on the pattern of use and value of Pertuzumab in real world settings. Our study describes the clinical efficacy and treatment costs of Pertuzumab in HER2-positive MBC treated in a tertiary cancer centre in Singapore in a predominantly Asian population.

AIM

To investigate the clinical efficacy and treatment costs of Pertuzumab in HER2-positive MBC in an Asian population in Singapore.

METHODS

A retrospective study of 304 HER2-positive MBC patients seen at National Cancer Centre Singapore between 2011-2017 was conducted. Demographic and clinical data were extracted from electronic medical records. Clinical characteristics and billing data of patients who received Pertuzumab were compared with those who did not.

RESULTS

Thirty-one (62.0%) of the fifty (16.4%) patients who received Pertuzumab as first-line therapy. With a median follow-up of 21.5 mo, there was a statistically significant difference in the median overall survival between Pertuzumab and

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non-Pertuzumab groups [51.5 (95%CI: 35.8–60.0) *vs* 32.9 (95%CI: 28.1–37.5) mo; *P* = 0.0128]. Two (4.88%) patients in the Pertuzumab group experienced grade 3 (G3) cardiotoxicity. The median treatment cost incurred for total chemotherapy for the Pertuzumab group was 130456 Singapore Dollars compared to 34523 Singapore Dollars for the non-Pertuzumab group. The median percentage of total chemotherapy costs per patient in the Pertuzumab group spent on Pertuzumab was 50.3%.

CONCLUSION
This study shows that Pertuzumab use in the treatment of metastatic breast cancer is associated with a significantly better survival and a low incidence of serious cardiotoxicity. However, the proportionate cost of Pertuzumab therapy remains high and further cost-effectiveness studies should be conducted.

Key words: Pertuzumab; Chemotherapy; Metastatic breast cancer; Treatment cost

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Core tip: Pertuzumab is a humanized anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibody found in a Phase III clinical trial to significantly improve median survival in HER2 positive metastatic breast cancer when used in combination with a taxane and Trastuzumab, and its clinical efficacy has transformed the therapeutic landscape of HER2-positive breast cancer. In our study, there was a statistically significant difference in the median overall survival between Pertuzumab and non-Pertuzumab groups [51.5 (95%CI: 35.8–60.0) *vs* 32.9 (95%CI: 28.1–37.5) mo; *P* = 0.0128] while the rate of grade 3 cardiotoxicity was low (4.88%). However, costs remain high.

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INTRODUCTION

Cancer is a leading cause of death worldwide, and has recently taken over cardiovascular disease as the leading cause of death in developed countries[1]. Majority of adults are concerned about contracting cancer, especially the biopsychosocial aspects that accompany the diagnosis[2]. According to the Singapore Cancer Registry, breast cancer is the most common female malignancy, accounting for 29.1% of all female cancers with an age-standardized incidence rate that has increased threefold since the 1970s. A national government-subsidized breast cancer screening program (BreastScreen Singapore) estimates 7% of new breast cancer cases in Singapore are metastatic at presentation. Metastatic breast cancer (MBC) remains a lethal disease with a historical 5-year survival rate of 22% or lower. However, median survival improved over the past 25 years[3] now ranging from 33[3] to 37 mo[4]. Human epidermal growth factor receptor 2 (HER2) overexpression is seen in 20% of breast cancers and follows a clinically aggressive course with poorer prognosis[5]. Pertuzumab is a humanized anti-HER2 monoclonal antibody found in a Phase III clinical trial to significantly improve median survival in HER2 positive MBC when used in combination with a taxane and Trastuzumab[6], and its clinical efficacy has transformed the therapeutic landscape of HER2-positive breast cancer[7]. Given its mortality benefit when added to a trastuzumab-based regimen as first-line therapy[8], it was approved by Singapore's health authority (Health Sciences Authority) in 2014. Despite its prevalence, the mortality and morbidity of cancer has improved dramatically with the advent of novel therapeutic options. Thus, our study aims to describe the clinical use, efficacy and costs of Pertuzumab in HER2 positive MBC treated in a tertiary cancer centre in Singapore.

MATERIALS AND METHODS

Patient selection

We retrieved electronic medical records of 1185 consecutive patients with newly diagnosed MBC referred to the Division of Medical Oncology of National Cancer Centre Singapore (NCCS) from 1st January 2011 to 31st December 2017 from the Joint Breast Cancer Registry. The study was reviewed and approved by the SingHealth Centralised Institutional Review Board Reference: 2018/2400.

Patients with histologically-proven breast cancer, radiological evidence of metastatic disease, and HER2 positivity on immunohistochemistry (IHC) or fluorescence *in situ* hybridization were selected. HER2 positivity was defined as a score of 3+ on IHC or an IHC score of 2+ and a HER2/CEP17 ratio ≥ 2.0 for samples after 1 January 2014 and HER2/CEP17 ratio ≥ 2.2 for samples before 1 January 2014 on fluorescence *in situ* hybridization testing.

After exclusion of 855 patients with HER2 negative and unknown HER2 status, clinical and treatment data were collected for 329 HER2 positive MBC patients. Patients with unknown Pertuzumab usage were then excluded, and data analysis was conducted for all 304 HER2 positive MBC patients. For further cost analyses, patients with incomplete billing data were excluded.

Source of data

Clinical data was retrieved in stages. In the first stage, patient demographics, diagnosis, date of death and clinical variables were retrieved electronically from the Department of Cancer Informatics of NCCS. Clinical variables included: Estrogen receptor (ER) status, progesterone receptor (PR) status, HER2 status, histological grade and subtype, diagnosis date, age of patient at diagnosis, TNM staging and site of metastases. Presence of visceral metastases was defined as metastases in the liver, lung or brain.

In the second stage, detailed treatment histories of each patient were constructed using the electronic medical records, supplemented by data from the NCCS MOSAIQ chemotherapy prescription database and pharmacy billing records in the SingHealth Electronic Health Intelligence System (eHINTS).

Data on the duration of treatment and date of progression was collected for every line of treatment. Duration of treatment was determined by the patients' dates of registered visits to the outpatient chemotherapy unit as well as from the outpatient records. The date of progression was obtained based on the date of radiological findings of progressive disease.

In patients who received Pertuzumab, treatment outcomes and order of line of therapy were also noted. This included documenting best response clinically or radiologically, as well as the presence of cardiotoxic side effects. Cardiotoxic side effects was defined as Grade 3 Left Ventricular Systolic Dysfunction by the presence of resting ejection fraction of 40%–50% or 10%–19% drop from baseline on two-dimensional echocardiography based on the Common Terminology Criteria for Adverse Events Version 4.03. Echocardiography and multiple-gated acquisition results of patients were systematically reviewed to asses for cardiotoxicity consistent with Common Terminology Criteria for Adverse Events Grade 3 Left Ventricular Systolic Dysfunction. Patients with no echocardiography, multiple-gated acquisition scan of the heart or clinical documentation were excluded from this analysis.

In the last stage, costs incurred by patients were retrieved based on inpatient and outpatient bills, including total treatment cost for chemotherapy and total treatment cost of Pertuzumab. Patients who had Pertuzumab treatment but did not have any corresponding bills were excluded from this analysis.

Statistical analysis

All the patients were categorized as "yes" or "no" based on status of Pertuzumab use. All demographic, clinical and histological data were summarized based on Pertuzumab use. Continuous and categorical variables were summarized as mean [standard deviation (SD), range] or median (inter-quartile range, range), whichever applicable, and frequency (percentage) respectively. Difference between statuses of Pertuzumab group was tested using 2-sample independent *t*-test or Mann-Whitney *U*–test, whichever applicable, and fisher's exact test for continuous and categorical data respectively.

We also plotted Kaplan-Meier curve to find a difference in overall survival (OS) between statuses of Pertuzumab use. We defined OS as duration between date of diagnosis to date of death or date of last follow up, whichever was later. Difference between Pertuzumab and non-Pertuzumab group was assessed using log-rank test and median survival time. Univariate and multivariable Cox proportional hazard regression model was used to find associated risk factors of OS this population.

Quantitative association from Cox regression was expressed as hazard ratio (HR) with its corresponding 95%CI. All the tests used in this study were two sided and *P* values < 0.05 was considered as statistical significance. All statistical analyses were carried out using SAS Institute Inc 2013. SAS/ACCESS® 9.4. Cary, NC: SAS Institute Inc.

RESULTS

Patient characteristics

The patient and disease characteristics of the study population are shown in Table 1. The mean ± SD age at MBC diagnosis was 58.1 (11.7) years. The majority of the patients were of Chinese ethnicity (64.5%). The median (interquartile range) duration of follow-up was 21.5 (27.8) mo.

The majority of patients had invasive ductal carcinoma (88.2%) while other histological subtypes comprised the remaining 11.8%. Most breast cancers were histological grade 3 (70.7%). Among the 304 patients, 297 (97.7%) were de novo metastatic cancers. The most common site of metastasis was to the bone, seen in 167 (54.9%) patients.

Descriptive statistics of study population

In this study population, 50 (16.4%) patients received Pertuzumab. Of these 50 patients, 31 (62.0%) patients had first-line Pertuzumab therapy. Patients who received Pertuzumab were significantly younger (54.5 years *vs* 58.9 years, *P* = 0.0133). There was also a significant difference in ethnic distribution with more patients of Indian and other ethnicities in the Pertuzumab group (*P* = 0.0003). There was no significant difference between hormone receptor status, grade, histology subtype and site of metastases.

Survival analysis

The Kaplan-Meier curve (Figure 1) showed statistically significant increase in OS among the patients who received Pertuzumab (*P* = 0.0128). The median OS of the Pertuzumab and non-Pertuzumab group were 51.5 (95%CI: 35.8–60.0) and 32.9 (95%CI: 28.1–37.5) mo respectively.

All the variables were analysed to find associated risk factors of overall survival (Table 2). Univariate regression analyses revealed that Pertuzumab usage (HR = 0.515, 95%CI: 0.303–0.877, *P* = 0.0145) and positive ER status (HR = 0.722, 95%CI: 0.531–0.981, *P* = 0.0374) were significantly associated with increased survival, while presence of liver (HR = 1.900, 95%CI: 1.395–2.590, *P* < 0.0001), lung (HR = 1.393, 95%CI: 1.023–1.897, *P* = 0.0352), and brain metastases (HR = 2.953, 95%CI: 1.844–4.730, *P* < 0.0001), were significantly associated with decreased survival. Although age and race of the subpopulations were different, they did not significantly affect survival.

Multivariate analysis was conducted to elucidate associations between Pertuzumab use, ER and PR status, and presence of brain, lung and liver metastases. It revealed that site of metastases (brain, liver, lung, bone) and Pertuzumab usage continued to be significantly associated with survival differences, while ER and PR difference did not result in statistically different survival outcomes.

Pertuzumab response and side effects

Two (4.88%) of the 41 patients who received Pertuzumab experienced grade 3 cardiotoxicity. Nine patients had unknown side effect status. Thirty-three (66.0%) patients achieved either complete or partial remission as best response to Pertuzumab therapy. Seven (14.0%) patients had unknown response status, while 5 (10%) patients had stable disease and 5 (10.0%) had progressive disease.

Treatment costs

For the study population, treatment costs of the chemotherapy were extracted in Singapore Dollars (SGD) and reviewed. The median cost on chemotherapy of the subgroup with Pertuzumab was higher at a median of SGD 130456 compared to SGD 34523 in the subgroup without Pertuzumab. Similarly, the median cost on all services incurred in NCCS of the subgroup with Pertuzumab was higher at a median of SGD 170875 compared to SGD 63741 in the subgroup without Pertuzumab. The median percentage of total chemotherapy costs and total services spent on Pertuzumab is 50.3% and 37.3%, respectively.

Table 1 Patient and disease characteristics

Characteristics	Pertuzumab usage		Total (*n* = 304)	*P* value
	Yes (*n* = 50)	No (*n* = 254)		
Age at MBC diagnosis				0.0133
Mean ± SD	54.5 ± 11.64	58.9 ± 11.53	58.1 ± 11.65	
Range	32–79	23–95	23–95	
Race, *n* (%)				0.0003
Chinese	29 (58.0)	167 (65.7)	196 (64.5)	
Malay	4 (8.00)	57 (22.4)	61 (9.21)	
Indian	5 (10.0)	14 (5.51)	19 (6.25)	
Others	12 (24.0)	16 (6.30)	28 (20.1)	
Hormone receptor status, *n* (%)				
ER positive	32 (64.0)	125 (49.2)	157 (51.6)	0.0638
PR positive	18 (36.0)	82 (32.2)	102 (33.6)	0.3264
Grade, *n* (%)				0.8160
Grade 1	0 (0.00)	3 (1.83)	3 (1.515)	
Grade 2	8 (23.53)	47 (28.7)	55 (27.8)	
Grade 3	26 (76.5)	114 (69.5)	140 (70.7)	
Histology, *n* (%)				0.0899
Invasive ductal carcinoma	48 (96.0)	220 (86.6)	268 (88.2)	
Others	2 (4.00)	34 (13.4)	36 (11.8)	
Site of metastases, *n* (%)				
Brain	6 (12.0)	17 (6.69)	23 (7.57)	0.2369
Lung	18 (36.0)	115 (45.3)	133 (43.8)	0.2754
Liver	22 (44.0	98 (38.6)	120 (39.5)	0.5276
Bone	30 (60.0)	137 (53.9)	167 (54.9)	0.4425
Other	16 (32.0)	94 (37.0)	110 (36.2)	0.5249
Follow-up duration (yr)				0.8511
Median (IQR)	20.6 (21.6)	22.5 (29.5)	21.5 (27.8)	
Range	1–74	0–92	0–92	

MBC: Metastatic breast cancer; ER: Estrogen receptor; PR: Progesterone receptor; IQR: Interquartile range.

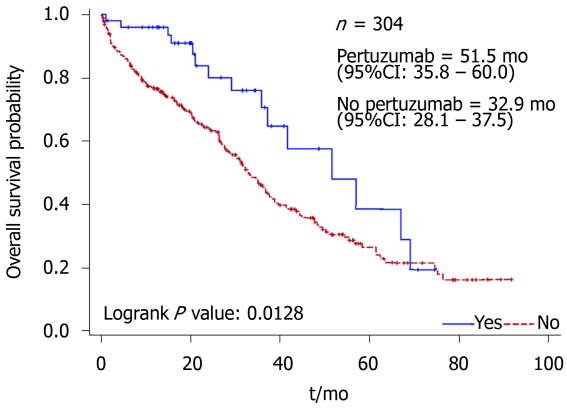
DISCUSSION

Current guidelines recommend the use of Pertuzumab, Trastuzumab and taxanes for the treatment of metastatic HER2 positive breast cancer. Dual HER2 blockade by adding Pertuzumab to Trastuzumab and a taxane backbone has significantly improved disease control, improved overall survival, duration to progression of disease and improved tumor response to therapy[9,10]. Usage of Pertuzumab as first line therapy was consistent with clinical guidelines, and has demonstrated reproducible safety and efficacy[11]. To further assess real-world efficacy of adding Pertuzumab to Trastuzumab and taxanes, we compared our study population with that of the CLEOPATRA[6] study in terms of clinical efficacy, side effects and cost effectiveness.

Comparison with CLEOPATRA trial

Clinical characteristics of this study was compared to the CLEOPATRA trial. The mean age of diagnoses was 58.1 for our study compared to 54 in the CLEOPATRA study. 55.3% of this study has ER and/or PR positive disease compared to 48.0% in the CLEOPATRA study. 68.4% of this study has visceral metastases compared to 78.0% in the CLEOPATRA study.

Notably, 96.4% of our study population were of Asian ethnicity compared with 32% in the CLEOPATRA study. Despite having a slightly inferior overall survival than the CLEOPATRA trial, we are able to conclude that Pertuzumab does significantly prolong overall survival in study with a predominantly Asian population. The cross-study comparison of complete or partial response was also remarkably similar (66% in our study *vs* 68.4% in CLEOPATRA).



Yes	50	45	29	19	10	6	4	2	0		
No	254	186	137	96	61	41	22	13	6	1	0

Figure 1 Kaplan-Meier survival curve comparing median overall survival between patients who had Pertuzumab treatment and who did not have Pertuzumab treatment.

The slight difference in overall survival could be explained by medical comorbidities of the different studies, difference in age, and also follow up duration. In addition, the CLEOPATRA study had a longer median follow-up duration of 50 mo compared to 21.5 mo in this study, and a younger population age of 54 compared to 58.1 in this study.

Prognostic factors

Consistent with previous studies, presence of visceral metastases (brain, liver and lung) had the strongest association with poorer prognosis[12]. Although ER and PR positivity have been reported to be associated with a better prognosis[13] and higher histologic grade is a poor prognostic factor[14], our univariate and multivariate analyses only demonstrated statistically insignificant trends. This could be attributed to insufficient power of our sample size as well as the possibility that HER2 positivity may contribute significantly more to the prognosis of this subpopulation of breast cancer patients as opposed to the aforementioned factors.

Serious adverse effects of dual HER2 therapy

One major adverse effect of HER2 therapy is cardiotoxicity. Studies on Pertuzumab have shown cardiotoxicities to be mainly asymptomatic left ventricular systolic dysfunction or symptomatic heart failure[15].

In our study population, 4.88% of patients who had undergone Pertuzumab treatment experienced significant cardiotoxic side effects. These findings in the real-world setting corroborate with rates in the CLEOPATRA trial and the JACOB[16] trial which reported 6.1% and 5.0% incidence of cardiotoxicity, respectively.

Cost effectiveness

Given that many guidelines recommend the use of Pertuzumab due to its efficacy, it would also be prudent to consider literature on value-based and cost effectiveness studies. The United Kingdom National Institute for Health and Care Excellence guidelines concluded that the Incremental Cost-Effective Ratio (ICER) of Pertuzumab exceeds the limit for cost-effective use of United Kingdom's National Health Service resources, without special considerations as life-extending treatment for patients with incurable disease. This is based on a maximum acceptable ICER of Great Britain Pound (GBP) 30000 per Quality-adjusted Life Years (QALY) gained in treating metastatic breast cancer. Similarly, the American Society of Clinical Oncology published a cost-effectiveness study of addition of Pertuzumab to docetaxel and trastuzumab for HER2 positive metastatic breast cancer. Although median survival was 56.9 mo with Pertuzumab and 39.4 mo without, it concluded that Pertuzumab in addition to Docetaxel and Trastuzumab is unlikely to be cost-effective, with 0% probability of cost-effectiveness at United States Dollar 100000 per QALY gained[17]. Lastly, a cost-effectiveness study of addition of Pertuzumab in combination with Trastuzumab and Docetaxel for HER2 positive metastatic breast cancer in Japan demonstrated that, at a higher limit of GBP 50000, it is still not cost effective as the

Table 2 Univariate and multivariate COX regression

Characteristics	Univariate analysis			Multivariable analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age	1.010	0.997 – 1.023	0.1330			
Pertuzumab use	0.515	0.303 – 0.877	0.0145	0.468	0.271 – 0.809	0.0066
Positive ER status	0.722	0.531 – 0.981	0.0374	0.875	0.601 – 1.274	0.4871
Positive PR status	0.744	0.534 – 1.037	0.0806	0.770	0.511 – 1.161	0.2136
Presence of metastases						
Brain	2.953	1.844 – 4.730	< 0.0001	3.620	2.229 – 5.879	< 0.0001
Lung	1.393	1.023 – 1.897	0.0352	1.520	1.107 – 2.086	0.0097
Liver	1.900	1.395 – 2.590	< 0.0001	2.051	1.495 – 2.815	< 0.0001
Bone	1.321	0.966 – 1.804	0.0808			
Other sites	1.231	0.895 – 1.693	0.2007			
Grade (Reference: Grade 1)						
Grade 2	3.616	0.494 – 26.463	0.2056			
Grade 3	2.799	0.388 – 20.202	0.3075			
Race (Reference: Chinese)						
Indian	1.184	0.636 – 2.204	0.5952			
Malay	1.350	0.931 – 1.957	0.1135			
Others	0.620	0.313 – 1.227	0.1701			

Six variables were used for multivariate analyses based on the sample size. They are estrogen receptor status, progesterone receptor status, Pertuzumab use, presence of brain, lung and liver metastases. ER: Estrogen receptor; PR: Progesterone receptor.

ICER of Pertuzumab addition is approximately GBP 90000 per QALY[18].

Given the above conclusions and the finding that Pertuzumab accounted for a median of 50.3% of total chemotherapy costs or a median cost of SGD 170875 over 21.5 mo in our study group, policymakers should conduct further cost-effectiveness studies to review the costs and funding models for Pertuzumab, and weigh the costs against the survival benefit. Further quality of life studies would also be helpful to assess the value of Pertuzumab for HER2-positive MBC.

Limitations

This retrospective study is subjected to limitations of studies based on electronic health records, which may contain incomplete information. However, efforts to corroborate information with multiple data sources from the Joint Breast Cancer Registry (JBCR) to databases containing chemotherapy prescriptions as well as billing data helped to minimize missing or discrepant data. In addition, this study was conducted at a single tertiary care cancer centre which may limit the extrapolation of data to other healthcare settings.

Conclusion

This study shows that Pertuzumab use in the treatment of metastatic breast cancer is associated with a significant improvement in survival benefit, without significant serious adverse effects associated with anti-HER2 agents. However, the proportionate cost of Pertuzumab therapy remains high and further cost-effectiveness studies should be conducted.

ARTICLE HIGHLIGHTS

Research background

Pertuzumab is an anti-HER2 agent that has demonstrated promising clinical efficacy in Phase III trials such as the CLEOPATRA trial. Given the incidence of breast cancer and the proportion of Stage IV breast cancers at diagnosis in an Asian population, there is a need to evaluate its efficacy in an Asian population, and review its costs.

Research motivation

We aim to study the use of Pertuzumab in National Cancer Centre Singapore in a predominantly Asian population.

Research objectives
We attempted to elucidate the clinical efficacy of Peruzumab in HER2-positive Metastatic breast cancer, evaluate the incidence of Grade 3 cardiotoxicity, and the costs of treatment. In so doing, we hope to guide policy makers on the use of Pertuzumab as an important arm of therapy for metastatic HER2-positive breast cancer.

Research methods
We systematically selected the patients based on inclusion criteria further described in the manuscript, and retrieved relevant clinical variables such as billing records, treatment history, patient demographics, response and side effects. Statistical analyses were conducted using SAS Institute Inc 2013.

Research results
This study demonstrated statistically significant difference in median overall survival favouring the Pertuzumab group, with low incidence of Grade 3 Cardiotoxicity. However, costs in the pertuzumab group remain significantly higher than the non-Pertuzumab group.

Research conclusions
We found that Pertuzumab had statistically significant survival benefit in an Asian population in Singapore. This study proposes that Pertuzumab should be adopted as first line therapy for HER2-positive metastatic breast cancer. To summarize the current knowledge, it supports the findings of CLEOPATRA trial, in an Asian population in Singapore. No similar study has been done in an Asian population in Singapore. The implications of this study is that further cost-effectiveness studies should be conducted on the usage of Pertuzumab.

Research perspective
This study demonstrated the clinical efficacy of Pertuzumab in an Asian population in Singapore, and serves as an impetus for future research on costs.

REFERENCES

1 **Yusuf S**, Joseph P, Rangarajan S, Islam S, Mente A, Hystad P, Brauer M, Kutty VR, Gupta R, Wielgosz A, AlHabib KF, Dans A, Lopez-Jaramillo P, Avezum A, Lanas F, Oguz A, Kruger IM, Diaz R, Yusoff K, Mony P, Chifamba J, Yeates K, Kelishadi R, Yusufali A, Khatib R, Rahman O, Zatonska K, Iqbal R, Wei L, Bo H, Rosengren A, Kaur M, Mohan V, Lear SA, Teo KK, Leong D, O'Donnell M, McKee M, Dagenais G. Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. *Lancet* 2019 [PMID: 31492503 DOI: 10.1016/S0140-6736(19)32008-2]

2 **Murphy PJ**, Marlow LAV, Waller J, Vrinten C. What is it about a cancer diagnosis that would worry people? A population-based survey of adults in England. *BMC Cancer* 2018; **18**: 86 [PMID: 29361912 DOI: 10.1186/s12885-017-3963-4]

3 **Sundquist M**, Brudin L, Tejler G. Improved survival in metastatic breast cancer 1985-2016. *Breast* 2017; **31**: 46-50 [PMID: 27810699 DOI: 10.1016/j.breast.2016.10.005]

4 **Gobbini E**, Ezzalfani M, Dieras V, Bachelot T, Brain E, Debled M, Jacot W, Mouret-Reynier MA, Goncalves A, Dalenc F, Patsouris A, Ferrero JM, Levy C, Lorgis V, Vanlemmens L, Lefeuvre-Plesse C, Mathoulin-Pelissier S, Petit T, Uwer L, Jouannaud C, Leheurteur M, Lacroix-Triki M, Cleaud AL, Robain M, Courtinard C, Cailliot C, Perol D, Delaloge S. Time trends of overall survival among metastatic breast cancer patients in the real-life ESME cohort. *Eur J Cancer* 2018; **96**: 17-24 [PMID: 29660596 DOI: 10.1016/j.ejca.2018.03.015]

5 **Arteaga CL**, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. *Nat Rev Clin Oncol* 2011; **9**: 16-32 [PMID: 22124364 DOI: 10.1038/nrclinonc.2011.177]

6 **Swain SM**, Baselga J, Kim SB, Ro J, Semiglazov V, Campone M, Ciruelos E, Ferrero JM, Schneeweiss A, Heeson S, Clark E, Ross G, Benyunes MC, Cortés J; CLEOPATRA Study Group. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med* 2015; **372**: 724-734 [PMID: 25693012 DOI: 10.1056/NEJMoa1413513]

7 **Slamon DJ**, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; **344**: 783-792 [PMID: 11248153 DOI: 10.1056/NEJM200103153441101]

8 **Dawood S**, Sirohi B. Pertuzumab: a new anti-HER2 drug in the management of women with breast cancer. *Future Oncol* 2015; **11**: 923-931 [PMID: 25760974 DOI: 10.2217/fon.15.7]

9 **Gradishar WJ**. Taxanes for the treatment of metastatic breast cancer. *Breast Cancer (Auckl)* 2012; **6**: 159-171 [PMID: 23133315 DOI: 10.4137/BCBCR.S8205]

10 **Gleeson JP**, Keegan NM, Morris PG. Adding Pertuzumab to Trastuzumab and Taxanes in HER2 positive breast cancer. *Expert Opin Biol Ther* 2018; **18**: 251-262 [PMID: 29183167 DOI: 10.1080/14712598.2018.1410132]

11 **Bachelot T**, Ciruelos E, Schneeweiss A, Puglisi F, Peretz-Yablonski T, Bondarenko I, Paluch-Shimon S, Wardley A, Merot JL, du Toit Y, Easton V, Lindegger N, Miles D; PERUSE investigators. Preliminary safety and efficacy of first-line pertuzumab combined with trastuzumab and taxane therapy for HER2-positive locally recurrent or metastatic breast cancer (PERUSE). *Ann Oncol* 2019; **30**: 766-773 [PMID: 30796821]

12 **Chen MT**, Sun HF, Zhao Y, Fu WY, Yang LP, Gao SP, Li LD, Jiang HL, Jin W. Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: a SEER population-based analysis. *Sci Rep* 2017; **7**: 9254 [PMID: 28835702 DOI: 10.1038/s41598-017-10166-8]

13 **Dunnwald LK**, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: a

prospective cohort of breast cancer patients. *Breast Cancer Res* 2007; **9**: R6 [PMID: 17239243 DOI: 10.1186/bcr1639]

14 **Rakha EA**, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, Blamey RW, Ellis IO. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. *J Clin Oncol* 2008; **26**: 3153-3158 [PMID: 18490649 DOI: 10.1200/JCO.2007.15.5986]

15 **Lenihan D**, Suter T, Brammer M, Neate C, Ross G, Baselga J. Pooled analysis of cardiac safety in patients with cancer treated with pertuzumab. *Ann Oncol* 2012; **23**: 791-800 [PMID: 21665955 DOI: 10.1093/annonc/mdr294]

16 **Tabernero J**, Hoff PM, Shen L, Ohtsu A, Shah MA, Cheng K, Song C, Wu H, Eng-Wong J, Kim K, Kang YK. Pertuzumab plus trastuzumab and chemotherapy for HER2-positive metastatic gastric or gastro-oesophageal junction cancer (JACOB): final analysis of a double-blind, randomised, placebo-controlled phase 3 study. *Lancet Oncol* 2018; **19**: 1372-1384 [PMID: 30217672 DOI: 10.1016/S1470-2045(18)30481-9]

17 **Durkee BY**, Qian Y, Pollom EL, King MT, Dudley SA, Shaffer JL, Chang DT, Gibbs IC, Goldhaber-Fiebert JD, Horst KC. Cost-Effectiveness of Pertuzumab in Human Epidermal Growth Factor Receptor 2-Positive Metastatic Breast Cancer. *J Clin Oncol* 2016; **34**: 902-909 [PMID: 26351332 DOI: 10.1200/JCO.2015.62.9105]

18 **Tange C**, Kunisawa S, Maeda S, Shimozuma K. Cost-Effectiveness Analysis Of Pertuzumab For Metastatic Her2-Positive Breast Cancer In Japan. *Value Health* 2015; **18**: A456-457 [DOI: 10.1016/j.jval.2015.09.1169]

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EVIDENCE-BASED MEDICINE

Assessment methods and services for older people with cancer in the United Kingdom

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Abstract

BACKGROUND

National cancer strategy calls for comprehensive assessments for older people but current practice across the United Kingdom is not well described.

AIM

To identify current assessment methods and access to relevant supporting services for older people with cancer.

METHODS

A web-based survey (SurveyMonkey) targeting health professionals (oncologists, cancer surgeons, geriatricians, nurses and allied health professionals) was distributed January-April 2016 *via* United Kingdom nationally recognised professional societies. Responses were analysed in frequencies and percentages. Chi Square was used to compare differences in responses between different groups.

RESULTS

640 health care professionals responded. Only 14.1% often/always involved geriatricians and 52.0% often/always involved general practitioners in assessments. When wider assessments were used, they always/often influenced decision-making (40.5%) or at least sometimes (34.1%). But 30.5%-44.3% did not use structured assessment methods. Most clinicians favoured clinical history taking. Few used scoring tools and few wished to use them in the future. Most had urgent access to palliative care but only a minority had urgent access to other key supporting professionals (*e.g.* geriatricians, social workers, psychiatry). 69.6% were interested in developing Geriatric Oncology services with geriatricians.

CONCLUSION

There is variability in assessment methods for older people with cancer across the United Kingdom and variation in perceived access to supporting services.

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Clinical history taking was preferred to scoring systems. Fostering closer links with geriatricians appears supported.

Key words: Geriatric assessment; Elderly; Older; Cancer; Support; Services

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Core tip: There was variability in assessment methods and access to supporting services for older people with cancer in the United Kingdom. Performance status and traditional history-taking was preferred to scoring tools. Future studies should consider moving away from scoring tools if the intention is for use in clinical practice. There was variability in access to key supporting services. Developing care pathways to better link up existing services would be helpful. Collaborative working with geriatricians appears supported. A number of questions remain. How can comprehensive geriatric assessment be feasibly embedded within cancer care pathways across a nation?

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INTRODUCTION

With longer life expectancy, patients presenting to cancer services are often older and often have complex multi-morbidity[1]. Such patients have poorer outcomes[2-4]. Cancer services were not designed to manage these complex patients. Models of care focus on early cancer detection, diagnosis, investigation and treatment[5]. There has been less emphasis on coordination of cancer care in the context of comorbidities and wider psychosocial and functional needs.

The United Kingdom Independent Taskforce report "Achieving World-Class Cancer Outcomes: A strategy for England 2015-2020" acknowledged this gap in national care pathways, and called for improved assessment methods for older people in the United Kingdom and better collaborative working with geriatricians[6]. The existing literature supports better co-ordinated interdisciplinary support to deliver geriatric assessment, manage comorbidity burden with the goal of improving outcomes[7]. Yet, so far, little has changed to embed system-wide comprehensive assessments into routine clinical care for older people in most cancer services in the United Kingdom.

Some European countries such as France have made more progress with national level financial investment in a Geriatric Oncology programme[8]. In France, they developed a standardised approach to identify vulnerable older patients[9]. They use a brief assessment tool (called the G8) as an initial assessment process for all patients aged over 75+ years[10]. Those identified as vulnerable by this tool are referred to onco-geriatricians for a more in-depth assessment. This avoids the cost of unnecessary in-depth assessment in "fit" older patients, whilst delivering a systematic approach to identifying those who are "less fit" and require more input[11]. They have demonstrated benefit of implementing such a national programme with better collaboration, better informed decision-making and provision of more comprehensive interventions to support older people undergoing cancer treatment.

The use of a brief assessment tools to case-find those requiring in-depth geriatric assessment (contrasting to clinical judgement/referral-based pathways) is the pathway of choice in most Geriatric Oncology services across the globe as well as in the literature. Although France uses the G8 as their tool of choice, there are a number of similar tools in the literature, largely showing similar feasibility as a method of identifying those who require further assessment and intervention[12-14]. The International Society of Geriatric Oncology reviewed these tools and came to the consensus that no tool was superior and services should use whatever tool is usable and feasible for them[15,16].

There is no current national Geriatric Oncology programme in the United Kingdom although pockets of good practice are emerging and some have been evaluated[17]. The extent of local change is not clearly understood and how much services have evolved

is unknown. The cancer strategy calls for redesign of care pathways for frailer older people, but it is important to understand where the gaps lie in order to design solutions compatible with existing patient needs, workforce and resources. This nationwide survey aimed to identify current assessment methods used for older people in United Kingdom cancer services and to identify current access to relevant supporting services.

MATERIALS AND METHODS

A web-based survey (SurveyMonkey) was distributed between January to April 2016 *via* United Kingdom nationally recognised professional societies (Association of Cancer Physicians, Royal College of Radiologists, British Geriatrics Society, the Association for Cancer Surgery, United Kingdom Oncology Nursing Society, Association of Chartered Physiotherapists in Oncology and Palliative Care, British Oncology Pharmacist Association and British Gynaecological Cancer society). The survey targeted relevant health professionals working with older people with cancer in the United Kingdom. This included oncologists, cancer surgeons, geriatricians, nurses and allied health professionals. Participation in the survey was voluntary.

The survey (available in supplement 1) consisted of 3 sections:

(1) Work background of respondents (including specialty, profession, hospital/practice); (2) Current assessment methods used when reviewing older patients including: Which professionals were involved in assessment; assessment methods and tools applied; age cut-offs for assessment; communication of assessments; influence of assessment on cancer treatment decision-making; and (3) Current access to relevant supporting services for older people; Which assessment methods they would consider to use the future.

The tools were electronically linked to the British Geriatrics Society Oncology Specialist Interest Group website as an information resource of the assessment methods discussed. The purpose of this was to introduce cancer healthcare providers to the options they may wish to trial as part of their assessment pathways in the future.

Questions were asked using a mixture of yes/no/don't know, five-point Likert scales and free text responses. Questionnaire validity was evaluated using a panel of experts (members of the United Kingdom Geriatric Oncology Expert Reference Group supported by Macmillan Cancer Support). This expert group included representatives from multiple relevant specialities (medical, clinical and acute oncology, geriatric medicine, surgery, general practitioners (GPs), health and social care researchers) and multiple professions (doctors, nurses, therapists, researchers) and older patient representatives. The panel reviewed the questionnaire for readibility, content and clarity of words. The content of the questionnaire was revised accordingly prior to dissemination. Ethical approval was obtained (IRAS no. 194929). Participant information was provided, including explanation that consent was implied through completion of the web-survey. Responses were analysed in frequencies and percentages. The denominator for percentages was the percentage of those who responded *(i.e.* excluding non-responders). Where different, the denominator has been described. Chi Square was used to compare the differences in responses between different groups. SPSS version 25 statistical software package (SPSS, Inc., Chicago, IL, United States) was used for data analysis.

RESULTS

640 health care professionals responded to the survey between January to April 2016. The backgrounds of respondents are summarised in Table 1, the largest represented group were oncologists.

Health professionals currently involved in assessment of older cancer patients

Table 2 summarises current multi-disciplinary involvement in patient care. Gaps in wider medical provision was demonstrated; only 14.1% often/always had geriatricians involved in the assessment of older people in cancer services, only 52.0% often/always involved GPs in the assessment. Most (89.4%) did routinely involve nurses but a significant minority rarely/never used social care or allied health professionals such as occupational therapy.

Current assessment methods used

Table 1 Background of respondents

Specialty	*n* (%)
Clinical oncology	135 (21.3)
Medical oncology	164 (25.8)
Cancer services/oncology other	93 (14.6)
Acute oncology	30 (4.7)
Haem-oncology	54 (8.5)
Surgery	49 (7.7)
Geriatrics/elderly care	61 (9.6)
Palliative care	23 (3.6)
Other	26 (4.1)
Discipline	
Doctor	332 (52.0)
Nurse	251 (39.3)
Physiotherapist/occupational therapist	34 (5.3)
Other	21 (3.3)
Tumour group	
All	241 (37.7)
Tumour specific	350 (54.7)
Not applicable	36 (5.6)

Represents percentage of those who responded excluding non-response.

Clinical history-taking and performance status were favoured assessment methods regardless of profession (Table 3). Scoring tools were used far less and were favoured by nurses more than doctors, $P < 0.0001$.

About 30.5%-44.3% of respondents did not use structured methods for assessment or assessment tools to evaluate patient factors such as comorbidities, function, falls or social background. Nurses were significantly more likely to use a structured assessment approach than doctors [$P < 0.001$ for all domains except medications review (0.046)].

Table 4 describes which specific tools are being used and explores potential interest in using existing tools in the future. The denominator for percentages was the total sample. Brief assessment tools from the Geriatric Oncology literature (G8, VES13) were rarely used with greater use of traditional tools such as Mini Mental State Examination, Abbreviated Mental Test, Malnutrition Universal Screening Tool and Body Mass Index. The Holistic Needs Assessment (HNA) was utilised by 44.8%. Clinical assessment was used to identify more complex patients by oncologists (54.2%), by CNSs (51.4%) or *via* multi-disciplinary team (MDT) meetings (44.4%), few used tools to case-find (20.9%). There was no clear preference for a specific tool.

Operationalising assessments and effect on decision-making

Most respondents reported not using an age cut-off for older people specific assessments in their clinical services ($n = 300$). Of those who did perform these assessments, 238 reported these assessments were communicated into the MDT, 208 in to clinic and 69 information/advice given *via* phone/email. 148 produced a structured letter/report from the assessment.

When performed, the majority reported these assessments always/mostly influenced cancer treatment decision-making (40.5%) or at least sometimes (34.1%).

Multi-disciplinary access

Table 5 summarises urgent access to supporting services. Most had urgent access to palliative care (78.5%) but only a minority had urgent access available to other key professionals such as geriatricians, social workers, old age psychiatry or to specialist older people nurses.

Dedicated geriatric oncology services

14.8% of respondents reported having a dedicated Geriatric Oncology service. Where they existed and respondents were aware of funding streams, they were most commonly funded through medicine directorates ($n = 20$) or temporary charity funding ($n = 20$) rather than through cancer, surgery or CCGs ($n = 3$, 6 and 1

Table 2 Health professionals involved in the assessment of older people presenting to cancer service (%)

Health professional	Always	Often	Sometimes	Rarely	Never	Don't know
Oncologist	51.4 (298/580)	36.9 (214/580)	7.1 (41/580)	2.2 (13/580)	1.6 (9/580)	0.1 (5/580)
Surgeon	19.4 (106/545)	34.1 (186/545)	34.3 (187/545)	5.1 (28/545)	3.5 (19/545)	3.5 (19/545)
Geriatrician	4.7 (25/531)	9.4 (50/531)	24.7 (131/531)	32.6 (173/531)	21.1 (112/531)	7.5 (40/531)
Nurse	59.3 (343/578)	30.1 (174/578)	5.9 (34/578)	1.4 (8/578)	1.4 (8/578)	1.9 (11/578)
Physiotherapist	7.6 (42/556)	28.2 (157/556)	36.3 (202/556)	15.8 (88/556)	5.8 (32/556)	6.3 (35//556)
Occupational therapist	6.1 (34/553)	28.9 (160/553)	33.5 (185/553)	19.0 (105/553)	6.1 (34/553)	6.3 (35/553)
Dietician	4.7 (26/554)	29.1 (161/554)	42.1 (233/554)	13.4 (74/554)	5.6 (31/554)	5.2 (29/554)
Social worker	2.9 (16/550)	19.1 (105/550)	41.6 (229/550)	19.8 (109/550)	8.9 (49/550)	7.6 (42/550)
Pharmacist	23.8 (131/550)	24.4 (134/550)	19.6 (108/550)	17.1 (94/550)	9.6 (53/550)	5.5 (30/550)
General practitioner	26.6 (143/538)	25.5 (137/538)	18.8 (101/538)	11.3 (61/538)	7.8 (42/538)	10.0 (54/538)

respectively). 69.6% of respondents were interested in developing services with geriatricians, only 5.5% did not support this concept, the remaining respondents were unsure.

DISCUSSION

This United Kingdom wide survey demonstrates variability in assessment methods and access to supporting services for older people with cancer across the United Kingdom. Overall, health professionals tended to prefer performance status and traditional history-taking. Encouragingly, more than half of professionals reported they already use structured assessment for assessing issues such as comorbidity, cognition and nutrition giving a good base to standardise practice across cancer services. Nurses were more likely to use a structured approach to assessment than doctors. Studies exploring the reasons behind these differences would be helpful. Efforts seem best suited to developing a collaborative model with doctors, nurses and others in providing these assessments within a team-based structure[18]. Geriatric Oncology services should be evaluated for clinical effectiveness and feasibility to ensure the desired collaborative care is achieved[19].

Validated scoring tools (including the G8 and frailty scores) were not often used and there was little appetite to use them in the future. There has been significant interest in the research community to discover the holy grail of clinical score or frailty tool to aid cancer treatment decision-making[15]. This survey demonstrates that front-line clinicians do not support this concept and favour clinical information. Front-line clinicians already use performance status, and it is likely they acknowledge that the wider issues are too complex to be reduced down to an additional numerical indicator of fitness. The findings of this survey would suggest that any tool applied in the United Kingdom should be brief and focus around clinical history to have clinician buy-in.

Work is required to better link cancer services with other generalist doctors. Few had geriatricians involved in the assessment of older people in cancer services, and only half involved GPs. Evidence suggests that there is currently variation in delivery of follow up cancer care by GPs[20]. Standardised assessment pathways should ensure clarity of the responsibilities of primary, secondary and tertiary care before, during and after cancer treatment.

Most respondents had nurses involved in assessment supporting the positive impact of previous investment in specialist cancer nurses who to an extent provide wider questioning through the HNA[21]. However, most acknowledge that although the HNA provides significant value, it does not provide the means for delivering comprehensive geriatric assessment. Reassuringly, almost all respondents reported access to palliative care services, the majority having urgent access demonstrating feasibility of early collaborative working with other services.

Sharing of assessments was variable. Only a minority used these assessments to inform the MDT meeting yet reported that assessments often influenced cancer treatment decision-making. This may be due to MDT meeting culture where decision-making is often diagnostics focussed often excluding patient-centre factors from MDT decision-making[22]. Lack of comorbidity and clinical information in MDT meetings hinder their effectiveness[23]. Evidence suggests that when comorbidity is identified post-MDT, patients are more likely to receive conservative treatment[22]. Future

Table 3 Assessment methods currently used (%)

	Used	Not used	Don't know
Assessment method			
Clinical history	98.5 (572/581)	1.0 (6/581)	0.5 (3/581)
Performance status	90.2 (513/569)	7.7 (44/569)	2.1 (12/569)
Scores	33.8 (179/529)	53.1 (281/529)	13.0 (69/529)
Local methods	13.7 (64/467)	69.0 (322/467)	17.3 (81/467)
Structured assessment and/or assessment tool used to assess			
Comorbidity	62.2 (345/555)	31.2 (173/555)	6.7 (37/555)
Medications	60.1 (333/554)	33.2 (184/554)	6.7 (37/554)
Frailty	46.8 (257/549)	44.3 (243/549)	8.9 (49/549)
Mobility	61.1 (337/552)	34.2 (189/552)	4.7 (26/552)
Falls	51.9 (285/549)	42.8 (235/549)	5.3 (29/549)
Function	65.0 (358/551)	30.5 (168/551)	4.5 (25/551)
Nutrition	58.5 (321/549)	35.2 (193/549)	6.4 (35/549)
Quality of life	52.8 (290/549)	40.1 (220/549)	7.1 (39/549)
Cognition	60.5 (329/544)	31.8 (173/544)	7.7 (42/544)
Mood	46.9 (254/542)	43.0 (233/542)	10.1 (55/542)
Social circumstances	57.2 (313/547)	36.2 (198/547)	6.6 (36/547)

Represents percentage of those who responded excluding non-response.

pathways delivering geriatric assessments need to consider how this information should feed into MDT decision-making or whether MDT meetings need radical change to allow for meaningful patient-centred discussion.

Access to key multidisciplinary team members was variable highlighting the lottery of use of supporting services across the United Kingdom. The survey was designed to scope clinical practice of individuals, including their access of these services rather than describe supporting service existence from a public health perspective. It is possible that more services exist than respondents were aware of and therefore did not access. Future work should focus on local service mapping to better link up existing services to avoid duplication given the workforce implications for developing new services[24]. This survey demonstrated that urgent geriatrician resource was sparse. Therefore, care models to deliver improved assessments must involve up-skilling cancer services. Developing intervention algorithms for cancer services to manage co-existing needs identified by geriatric assessment should be tested. Feasibility of such algorithms using technology has been evaluated elsewhere[25]. This would reduce the need for geriatrician input for only the most complex.

Some geriatric oncology services were operational but surprisingly were funded more often through medicine directorate investments or charities. This contrasts to countries such as France where strong national investment has been applied[8]. Most were supportive of developing Geriatric Oncology services demonstrating a willingness for change. Similar previous surveys of Chiefs of Medical Oncology divisions in Italy were equally supportive of more time, attention and resource to older cancer patients[26].

This is the first survey to describe assessment methods used by front-line clinicians working in United Kingdom cancer services. This data is important in beginning to understand what clinicians in the United Kingdom will or won't buy into. It was completed by professionals working in cancer services with sufficient representation from different groups (medical and clinical oncology, surgery, nursing and therapies) to gain a sense of views and clinical practice.

However, the survey has limitations. Participation in the survey was voluntary and it was distributed widely through professional societies, therefore we cannot evaluate response rates. It is likely that those more motivated to improving older people care were more likely to respond thus may not be representative of all front-line staff. The survey was designed to allow respondents to skip any question they wished to. This resulted in some questions having more missing data than others. We cannot make any conclusions as to why questions were skipped by some respondents. We would propose that some may relate to lack of awareness of local service availability. The questionnaire was only internally validated. It would benefit from external validation to exclude problems with the survey design. We only collected basic data on the

Table 4 Specific tools used or would consider to use, % (*n*)

	Used	Would consider to use but currently do not use
ASA score	10.5 (67)	3.9 (25)
POSSUM score	5.9 (38)	4.7 (30)
G8 score	1.7 (11)	9.2 (59)
VES13	0.6 (4)	5.2 (33)
ACE27	4.8 (31)	8.1 (52)
CCI	6.7 (43)	12.8 (82)
Number of comorbidities	19.4 (124)	
Barthel's index of ADLs	15.0 (96)	12.0 (77)
iADLs	15.2 (97)	8.6 (55)
AMT score	27.3 (175)	6.1 (39)
MMSE	36.7 (235)	7.7 (49)
MoCA	7.5 (48)	3.9 (25)
GDS	6.9 (44)	13.6 (87)
HADS	20.2 (129)	8.9 (57)
PHQ9	2.5 (16)	9.1 (58)
MUST	32.8 (210)	8.3 (53)
BMI	56.1 (359)	5.8 (37)
EFS	3.8 (24)	13.1 (84)
GFI	0.0 (1)	8.4 (54)
Rockwood pictorial frailty scale	1.4 (9)	8.8 (56)
HNA	44.8 (287)	11.3 (72)
EORTC QLQ-C30	9.8 (63)	11.4 (73)
CGA-GOLD questionnaire	Not asked as not well known	8.1 (52)

Percentage of all participants. ASA: American Society of Anaesthesiologists; POSSUM: Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity; G8: Geriatric 8; VES13: Vulnerable Elders Survey; ACE27: Adult Comorbidity Evaluation 27; CCI: Charlson Comorbidity Index; ADLs: Activities of Daily Living; iADLs: Instrumental Activities of Daily Living; AMT: Abbreviated Mental Test; MMSE: Mini Mental State Examination; MoCA: Montreal Cognitive Assessment; GDS: Geriatric Depression Scale; HADS: Hospital Anxiety and Depression Scale; PHQ9: Patient Health Questionnaire–9; MUST: Malnutrition Universal Screening Tool; BMI: Body Mass Index; EFS: Edmonton Frailty Scale; GFI: Groningen Frailty Indicator; HNA: Holistic Needs Assessment; EORTC QLQ-C30: European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30; CGA-GOLD: Comprehensive Geriatric Assessment – Geriatric Oncology Liaison Development.

background of respondents. Future work should consider collecting more baseline characteristics of the respondents to allow for multivariate analysis of factors associated with different assessment preferences.

There is variability in assessment methods for older people with cancer across the United Kingdom and variation in perceived access to supporting services. Clinical history taking was preferred to scoring systems. Fostering closer links with geriatricians appears supported.

Table 5 Multi-disciplinary access (%)

Health professional	Urgent access	Routine access	No access	Don't know
Geriatrician	24.8 (118/475)	48.0 (228/475)	19.4 (92/475)	7.8 (37/475)
Medical physician	41.3 (191/462)	37.2 (172/462)	16.2 (75/462)	5.2 (24/462)
Single organ specialists	43.6 (203/466)	44.2 (206/466)	7.5 (35/466)	4.7 (22/466)
General practitioners	39.9 (188/471)	49.5 (233/471)	7.2 (34/471)	3.4 (16/471)
Physiotherapists	42.9 (204/476)	47.1 (224/476)	5.9 (28/476)	4.2 (20/476)
Occupational therapists	37.2 (178/479)	52.0 (249/479)	6.1 (29/479)	4.8 (23/479)
Dietician	38.9 (186/478)	54.0 (258/478)	4.2 (20/478)	2.9 (14/478)
Social workers	24.7 (116/470)	58.9 (277/470)	9.8 (46/470)	6.6 (31/470)
Psychological	26.5 (126/475)	56.8 (270/475)	11.8 (56/475)	4.9 (23/475)
Older peoples nurse	17.3 (82/473)	31.3 (148/473)	33.6 (159/473)	17.8 (84/473)
Psychiatry	16.5 (78/473)	47.1 (223/473)	21.8 (103/473)	14.6 (69/473)
Palliative care	78.7 (377/479)	19.2 (92/479)	0.6 (3/479)	1.5 (7/479)

ARTICLE HIGHLIGHTS

Research background

The United Kingdom Independent Taskforce report "Achieving World-Class Cancer Outcomes" calls for improved assessment methods for older people. Existing evidence and international bodies such as the American Society of Clinical Oncology and the International Society of Geriatric Oncology support this concept and recommend routine comprehensive geriatric assessment. However, assessment methods across a nation have yet to be described.

Research motivation

Older patients with cancer often have complex multi-morbidity and wider needs. These patients have poorer outcomes and are less likely to receive curative treatment. By better understanding current clinical assessment methods, future clinical care pathways can be designed around gaps in practice and be evaluated for effectiveness.

Research objectives

This nationwide survey aimed to identify current assessment methods and access to relevant supporting services for older people with cancer. By understanding current clinical practice and views, future research can focus towards interventions likely to be most acceptable and useful.

Research methods

A web-based survey was distributed between January to April 2016 *via* United Kingdom nationally recognised professional societies. The survey targeted relevant health professionals working with older people with cancer in the United Kingdom.

Research results

There was variability in assessment methods and access to supporting services for older people with cancer in the United Kingdom. Health professionals preferred performance status and traditional history-taking to scoring tools. Few had geriatricians involved in the assessment of older people and only half involved general practitioners. Access to key multidisciplinary team members was variable. This is the first study to describe assessment methods used by front-line clinicians in the United Kingdom. This data is important to informing design of future services to improve clinical assessment and support for older people with cancer.

Research conclusions

There was variability in assessment methods and access to supporting services. Clinical history taking was preferred to scoring systems. Future research evaluating delivery of comprehensive geriatric assessment should bear these results in mind. Future studies should consider moving away from scoring tools if the intention is for use in clinical practice. Developing care pathways to better link up existing supporting services would be a helpful initial step to improve access to key other professionals.

Research perspectives

A number of questions remain. How can comprehensive geriatric assessment be feasibly embedded within cancer care pathways across a nation? Is the workforce adequately trained to managed co-existing needs alongside cancer treatment? If not, would changes in education provide cancer services with the skills to better manage complex older patients? Or is collaborative working more effective? How can we test new assessment methods for feasibility and clinical effectiveness in cancer services?

REFERENCES

1 **Smith AW**, Reeve BB, Bellizzi KM, Harlan LC, Klabunde CN, Amsellem M, Bierman AS, Hays RD. Cancer, comorbidities, and health-related quality of life of older adults. *Health Care Financ Rev* 2008; **29**: 41-56 [PMID: 18773613 DOI: 10.1007/s11764-016-0553-4]

2 **Jørgensen TL**, Hallas J, Friis S, Herrstedt J. Comorbidity in elderly cancer patients in relation to overall and cancer-specific mortality. *Br J Cancer* 2012; **106**: 1353-1360 [PMID: 22353805 DOI: 10.1038/bjc.2012.46]

3 **Quaglia A**, Capocaccia R, Micheli A, Carrani E, Vercelli M. A wide difference in cancer survival between middle aged and elderly patients in Europe. *Int J Cancer* 2007; **120**: 2196-2201 [PMID: 17285582 DOI: 10.1002/ijc.22515]

4 **Verdecchia A**, Francisci S, Brenner H, Gatta G, Micheli A, Mangone L, Kunkler I; EUROCARE-4 Working Group. Recent cancer survival in Europe: a 2000-02 period analysis of EUROCARE-4 data. *Lancet Oncol* 2007; **8**: 784-796 [PMID: 17714993 DOI: 10.1016/S1470-2045(07)70246-2]

5 **The NHS Long Term Plan**. NHS England, 2019. Available from: https://www.longtermplan.nhs.uk/publication/nhs-long-term-plan/

6 **The Independent Cancer Taskforce 2015**. Achieving World-Class Cancer Outcomes: A Strategy for England 2015-2020. Available from: https://www.england.nhs.uk/wp-content/uploads/2017/10/national-cancer-transformation-programme-2016-17-progress.pdf

7 **Tremblay D**, Charlebois K, Terret C, Joannette S, Latreille J. Integrated oncogeriatric approach: a systematic review of the literature using concept analysis. *BMJ Open* 2012; 2 [PMID: 23220777 DOI: 10.1136/bmjopen-2012-001483]

8 **Antoine V**, de Wazières B, Houédé N. [Professional's expectations to improve quality of care and social services utilization in geriatric oncology]. *Bull Cancer* 2015; **102**: 150-161 [PMID: 25649352 DOI: 10.1016/j.bulcan.2014.12.011]

9 **Sifer-Rivière L**, Saint-Jean O, Gisselbrecht M, Cudennec T, Girre V; Programme d'OncoGériatrie de l'Ouest Parisien (POGOP). What the specific tools of geriatrics and oncology can tell us about the role and status of geriatricians in a pilot geriatric oncology program. *Ann Oncol* 2011; **22**: 2325-2329 [PMID: 21325447 DOI: 10.1093/annonc/mdq748]

10 **Bellera CA**, Rainfray M, Mathoulin-Pélissier S, Mertens C, Delva F, Fonck M, Soubeyran PL. Screening older cancer patients: first evaluation of the G-8 geriatric screening tool. *Ann Oncol* 2012; **23**: 2166-2172 [PMID: 22250183 DOI: 10.1093/annonc/mdr587]

11 **Soubeyran P**, Bellera C, Goyard J, Heitz D, Curé H, Rousselot H, Albrand G, Servent V, Jean OS, van Praagh I, Kurtz JE, Périn S, Verhaeghe JL, Terret C, Desauw C, Girre V, Mertens C, Mathoulin-Pélissier S, Rainfray M. Screening for vulnerability in older cancer patients: the ONCODAGE Prospective Multicenter Cohort Study. *PLoS One* 2014; **9**: e115060 [PMID: 25503576 DOI: 10.1371/journal.pone.0115060]

12 **Hurria A**, Gupta S, Zauderer M, Zuckerman EL, Cohen HJ, Muss H, Rodin M, Panageas KS, Holland JC, Saltz L, Kris MG, Noy A, Gomez J, Jakubowski A, Hudis C, Kornblith AB. Developing a cancer-specific geriatric assessment: a feasibility study. *Cancer* 2005; **104**: 1998-2005 [PMID: 16206252 DOI: 10.1002/cncr.21422]

13 **Ingram SS**, Seo PH, Martell RE, Clipp EC, Doyle ME, Montana GS, Cohen HJ. Comprehensive assessment of the elderly cancer patient: the feasibility of self-report methodology. *J Clin Oncol* 2002; **20**: 770-775 [PMID: 11821460 DOI: 10.1200/JCO.2002.20.3.770]

14 **Molina-Garrido MJ**, Guillén-Ponce C. Development of a cancer-specific Comprehensive Geriatric Assessment in a University Hospital in Spain. *Crit Rev Oncol Hematol* 2011; **77**: 148-161 [PMID: 20353897 DOI: 10.1016/j.critrevonc.2010.02.006]

15 **Wildiers H**, Heeren P, Puts M, Topinkova E, Janssen-Heijnen ML, Extermann M, Falandry C, Artz A, Brain E, Colloca G, Flamaing J, Karnakis T, Kenis C, Audisio RA, Mohile S, Repetto L, Van Leeuwen B, Milisen K, Hurria A. International Society of Geriatric Oncology consensus on geriatric assessment in older patients with cancer. *J Clin Oncol* 2014; **32**: 2595-2603 [PMID: 25071125 DOI: 10.1200/JCO.2013.54.8347]

16 **Decoster L**, Van Puyvelde K, Mohile S, Wedding U, Basso U, Colloca G, Rostoft S, Overcash J, Wildiers H, Steer C, Kimmick G, Kanesvaran R, Luciani A, Terret C, Hurria A, Kenis C, Audisio R, Extermann M. Screening tools for multidimensional health problems warranting a geriatric assessment in older cancer patients: an update on SIOG recommendations†. *Ann Oncol* 2015; **26**: 288-300 [PMID: 24936581 DOI: 10.1093/annonc/mdu210]

17 **Department of Health and Social Care**. Cancer Services Coming of Age report. 2012. Available from: https://www.gov.uk/government/publications/improving-older-peoples-access-to-cancer-treatment-services

18 **Caricati L**, Mancini T, Sollami A, Bianconcini M, Guidi C, Prandi C, Silvano R, Taffurelli C, Artioli G. The role of professional and team commitments in nurse-physician collaboration. *J Nurs Manag* 2016; **24**: E192-E200 [PMID: 26172247 DOI: 10.1111/jonm.12323]

19 **Shaw J**, Sethi S, Vaccaro L, Beatty L, Kirsten L, Kissane D, Kelly B, Mitchell G, Sherman K, Turner J. Is care really shared? A systematic review of collaborative care (shared care) interventions for adult cancer patients with depression. *BMC Health Serv Res* 2019; **19**: 120 [PMID: 30764822 DOI: 10.1186/s12913-019-3946-z]

20 **Meiklejohn JA**, Mimery A, Martin JH, Bailie R, Garvey G, Walpole ET, Adams J, Williamson D, Valery PC. The role of the GP in follow-up cancer care: a systematic literature review. *J Cancer Surviv* 2016; **10**: 990-1011 [PMID: 27138994 DOI: 10.1007/s11764-016-0545-4]

21 **Henry R**, Hartley B, Simpson M, Doyle N. The development and evaluation of a holistic needs assessment and care planning learning package targeted at cancer nurses in the UK. *Ecancermedicalscience* 2014; **8**: 416 [PMID: 24761157 DOI: 10.3332/ecancer.2014.416]

22 **Stairmand J**, Signal L, Sarfati D, Jackson C, Batten L, Holdaway M, Cunningham C. Consideration of comorbidity in treatment decision making in multidisciplinary cancer team meetings: a systematic review. *Ann Oncol* 2015; **26**: 1325-1332 [PMID: 25605751 DOI: 10.1093/annonc/mdv025]

23 **Jalil R**, Ahmed M, Green JS, Sevdalis N. Factors that can make an impact on decision-making and decision implementation in cancer multidisciplinary teams: an interview study of the provider perspective. *Int J Surg* 2013; **11**: 389-394 [PMID: 23500030 DOI: 10.1016/j.ijsu.2013.02.026]

24 **Bridges J**, Simcock R. Meeting the workforce challenges for older people living with cancer. *Int J Nurs*

Stud 2017; **65**: A1-A2 [PMID: 27884390 DOI: 10.1016/j.ijnurstu.2016.11.013]
25 **Loh KP**, Ramsdale E, Culakova E, Mendler JH, Liesveld JL, O'Dwyer KM, McHugh C, Gilles M, Lloyd T, Goodman M, Klepin HD, Mustian KM, Schnall R, Mohile SG. Novel mHealth App to Deliver Geriatric Assessment-Driven Interventions for Older Adults with Cancer: Pilot Feasibility and Usability Study. *JMIR Cancer* 2018; **4**: e10296 [PMID: 30373733 DOI: 10.2196/10296]
26 **Monfardini S**, Pasetto L, Jirillo A, Delai N; other participants. Taking care of older cancer patients: results of a survey addressed to the Chiefs of the Medical Oncology Divisions in Italy. *Crit Rev Oncol Hematol* 2006; **58**: 53-59 [PMID: 16531063 DOI: 10.1016/j.critrevonc.2005.08.004]

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CASE REPORT

Thrombocytopenia with multiple splenic lesions - histiocytic sarcoma of the spleen without splenomegaly: A case report

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Abstract

BACKGROUND

Histiocytic sarcoma (HS) of the spleen is reported to be a rare and lethal disease. The clinicopathological features of splenic HS have not been well described. The objective of this paper is to describe the diagnosis and treatment of a case of this rare disease and provide a review of the literature.

CASE SUMMARY

In this article, we discuss the case of a 40-year-old Hispanic female who presented with progressive thrombocytopenia and multiple hypoechoic lesions in the spleen without splenomegaly. Positron emission tomography-computed tomography showed increased activity in cervical lymph nodes, as well as multiple bone and splenic lesions with positive uptake. Two bone marrow biopsies and fine-needle aspiration of the cervical lymph node were inconclusive. Laparoscopic splenectomy was performed, and gross examination showed a 110.1 g spleen with multiple rubbery, nodular lesions within the subcapsular sinus and splenic parenchyma. The microscopic findings showed multinodular histiocyte proliferation with atypia and multilobulated nuclei, which were positive for CD163, CD4, and CD68 by immunohistochemical analysis. The final pathologic diagnosis was difficult and was found to be low-grade HS of the spleen, after consultations with two renowned hematopathology institutions. At the patient's five-month follow-up visit, her bone marrow metastasis had progressed. She is waiting to be enrolled in a clinical trial.

CONCLUSION

Pathologic diagnosis of splenic HS can be challenging. Low-grade differentiation

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may be associated with a slow progressive disease.

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Core tip: Histiocytic sarcoma (HS) of the spleen is a rare and potentially lethal condition. We report a patient with thrombocytopenia of unknown etiology, multiple splenic lesions, and disseminated bone metastasis treated by laparoscopic splenectomy. The final pathologic diagnosis showed low-grade HS of the spleen. It is very rare that HS occurs without splenomegaly.

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INTRODUCTION

Histiocytic sarcoma (HS) is a rare malignant neoplasm that occurs in lymph nodes, skin, and the gastrointestinal tract. It is a malignant proliferation of cells showing morphologic and immunophenotypic characteristics of mature tissue histiocytes[1], and represents less than 0.5% of all non-Hodgkin's lymphoma. HS of the spleen is a rare and potentially lethal condition that can remain asymptomatic or only mildly symptomatic for an extended period[2]. The diagnosis depends on verification of histiocytic lineage and distinguishing HS from other benign or malignant diseases, such as benign histiocytic proliferation, hemophagocytic syndrome, malignant histiocytosis, and acute monocytic leukemia using immunohistochemical techniques and molecular genetic tools[3-5].

Due to its rarity, the number of "true" cases reported as primary splenic HS in the English literature are few (up to eight cases reported), with most recent references from 2012. The clinicopathological features of HS have not been well described. Primary splenic HS cases often show a multi-nodular lesion in an enlarged spleen, with no specific findings in imaging studies[6,7]. Patients with splenic HS have a poor prognosis due to the aggressive behavior of this entity, even though a splenectomy might induce temporary remission. Liver or bone marrow infiltration constitutes a common finding that partially obscures the prognostic evaluation. Early evaluation and diagnosis, before dissemination of the disease, may improve the prognosis and prospects of survival. We report a case of thrombocytopenia of unknown etiology with multiple splenic lesions and disseminated bone metastasis treated by laparoscopic splenectomy. The final diagnosis was low-grade HS of the spleen.

CASE PRESENTATION

Chief complaints

Left upper quadrant pain, drenching sweats.

History of present illness

A 40-year-old Hispanic female was referred to our clinic due to persistent thrombocytopenia and multiple splenic lesions. She initially presented with drenching sweats and left upper abdominal pain that was associated with progressive thrombocytopenia for four months. She denied recent weight loss, fever, or chills.

History of past illness

The patient denied previous medical or surgical history.

Personal and family history

Not contributory.

Physical examination upon admission

On physical examination, no hepatomegaly, splenomegaly, or superficial lymphadenopathy was observed.

Laboratory examinations

Initial assessment revealed thrombocytopenia with a platelet count of 37000/mm^3. Her white blood cell count was 10300/μL, and hemoglobin was 14.0 g/dL. The biochemistry profile was unremarkable. Further study of serum protein electrophoresis, peripheral blood flow cytometry, and fine-needle aspiration (FNA) of the right cervical level II lymph node was unremarkable. She had two bone marrow biopsies, which did not show evidence of lymphoproliferative disorder.

Imaging examinations

Magnetic resonance imaging (MRI) of the spleen demonstrated a normal-sized spleen (greatest dimension 10 cm) with multiple T1 hypointense/T2 hyperintense lesions, suggestive of possible extramedullary hematopoiesis or lymphoma. Positron emission tomography-computed tomography (PET/CT) showed mild uptake (standardized uptake value (SUV) 3.5) in one of the lesions along the splenic hilum. All other splenic nodules were non-avid. A small cervical node (SUV 3.3), left breast nodule (SUV 2.1), and several bone sites (brightest at T3, SUV 4.3) were also noted.

FINAL DIAGNOSIS

Low-grade HS of spleen.

TREATMENT

Diagnostic assessment and treatment

The patient was started on 40 mg of prednisone. This was progressively increased to 80 mg daily, with some improvement in her platelet count, which had been stable at 75000/mm^3. She was otherwise doing well. She had no complaints of fever or weight loss, and no enlarged lymph nodes or neurological symptoms were noted on physical examination. The case was discussed at a tumor board. A decision was made to perform laparoscopic splenectomy. The patient was placed on a steroid taper with prednisone, 20 mg daily, preoperatively. Subsequently, her platelet count was hovering around 41000/mm^3.

During surgery, a 10 mm trocar was inserted at the mid-clavicular line between the umbilicus and the left costal margin, and three trocars (10 mm, 5 mm, 5 mm) were placed at the anterior axillary line and midline during the procedure. A laparoscopic view showed a normal-sized liver and spleen, with no accessory splenules visualized. In addition, no metastatic or disseminated lesions were detected. The spleen was extracted from a 4 cm midline incision. The operation time was 2 h, and blood loss was 150 mL. To address her thrombocytopenia, 20 U of platelets were transfused after splenic vessels were transected.

Pathological findings

Gross specimen: The weight of the resected spleen was 110.1 g and measured 12 cm × 8.0 cm × 3.6 cm. There were numerous nodular, tan, rubbery, well-circumscribed lesions with smooth surfaces. The lesions were located within the subcapsular sinus and splenic parenchyma (greater than ten lesions).

Microscopically, the H and E-stained sections showed a spleen with multinodular proliferation of histiocytoid cells. There was an accompanying infiltrate of scattered small lymphoid cells, neutrophils, and eosinophils. The histiocytoid cells had abundant eosinophilic cytoplasm and showed variable nuclear atypia (Figures 1 and 2). Immunohistochemistry showed immunoreactivity for CD163, CD4, and CD68, factor XIIIa, and CD14. The Ki-67 showed a proliferative index of 10%. Only rare mitotic figures, with rare atypical mitoses, were noted. The patient's pathologic diagnosis was difficult. After consulting two renowned pathology institutions, the final pathologic diagnosis was HS of the spleen.

OUTCOME AND FOLLOW UP

The postoperative course was uneventful. The patient's platelet count normalized after surgical intervention: 13900/mm^3 on postoperative day one (POD1) and

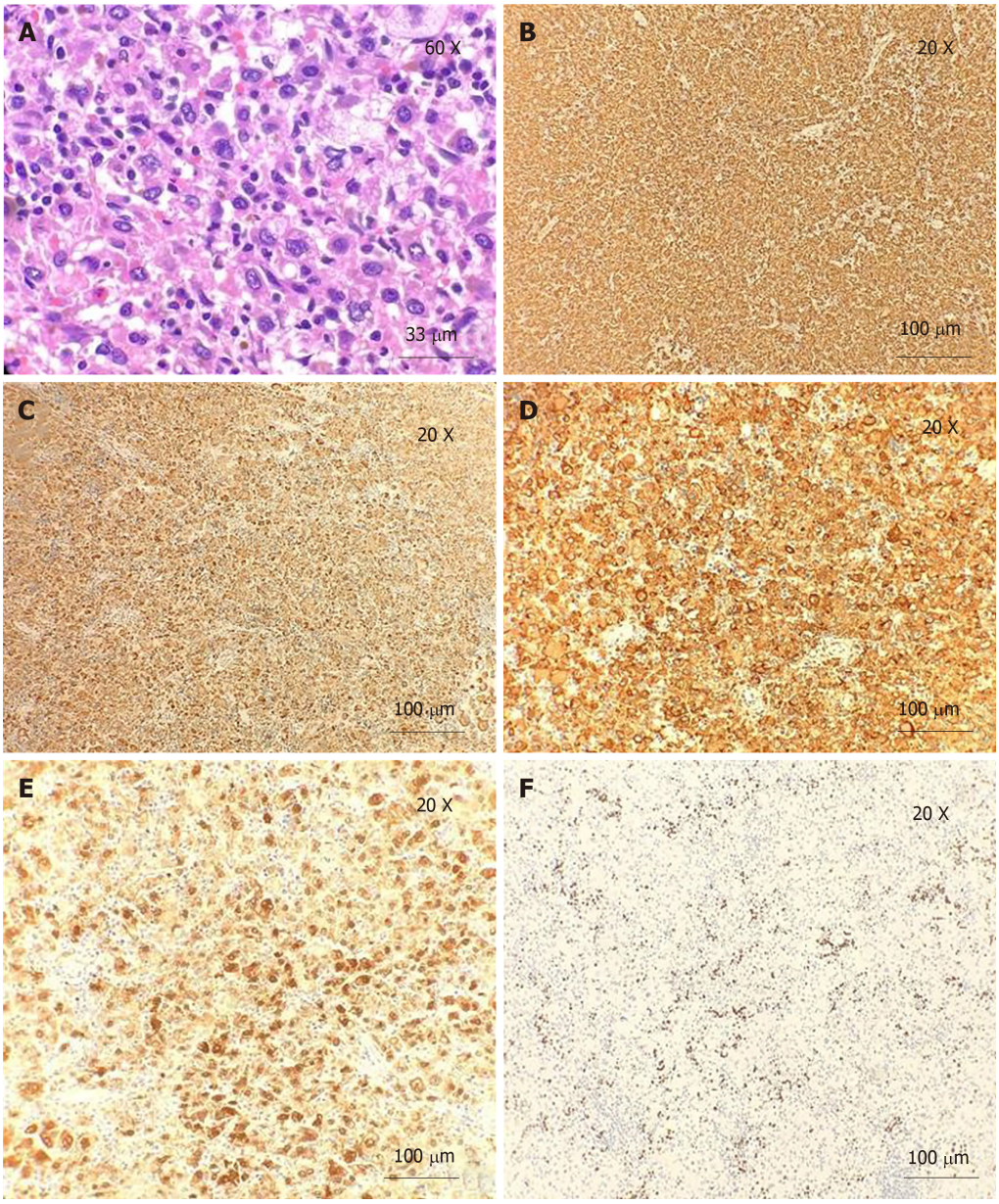

Figure 1 H and E staining and immunohistochemistry. A: H and E staining. Neoplastic cells have abundant eosinophilic and vacuolated cytoplasm, with a large eccentric nucleus and prominent nucleoli (60 × magnification); B: Immunoreactivity for CD163 (20 × magnification); C: Immunoreactivity for CD68 (20 × magnification); D: Immunoreactivity for CD4 (20 × magnification); E: Immunoreactivity for Factor XIII (20 × magnification); F: Ki-67. Proliferation index is approximately 10% (20 × magnification).

16300/mm^3 on POD2. The patient was discharged on POD2 with instructions to follow-up one month after surgery. She has been feeling well and had marked improvement in pain and night sweats, with platelet values remaining stable. She has not noticed any enlarged lymph nodes, fever, weight loss, or neurological deficits. The patient was seen at a renowned cancer center after a final diagnosis of low-grade HS. Her repeat PET, one month after surgery, showed low avidity of bone marrow without interval changes. She remains in observation without adjuvant treatment. However, her five-month PET scan showed progressed bone marrow metastasis. She is waiting to be enrolled in a clinical trial.

DISCUSSION

HS is a rare malignant proliferation of cells showing morphologic and immunophenotypic features, similar to mature tissue histiocytes. Clinically, it is generally accepted that most patients with HS have a poor prognosis due to early disseminated disease and limited response to chemotherapy[8]. Previous cases, including extra-nodal HS of non-splenic origin, showed that the stage of the disease and possibly tumor size

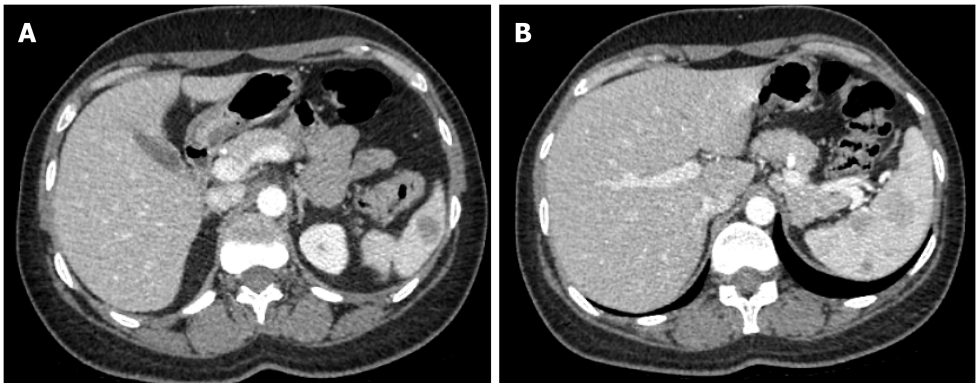

Figure 2 Computed tomography. A, B: Spleen shows multiple circumscribed intermediate density lesions of variable size and varying enhancement, with the largest at the hilum measuring 2.4 cm × 2.1 cm and 2.7 cm × 2.1 cm. The differential diagnosis includes lymphoma, splenic peliosis, infection with multiple abscesses, and metastasis.

can be important prognostic indicators[9,10]. HS of the spleen is a rare condition, and its clinicopathological features have not been well described[7]. According to the nine cases reported so far (Table 1), the majority of patients are female (6/9), with an age range of 29-82 years (median 67), and presented with thrombocytopenia and multinodular lesions in the setting of splenomegaly. The most common distant metastases are liver and bone marrow. Survival time ranges from 1 month to 5 years (median six months). Early diagnosis, before dissemination of this disease, may improve the prognosis. Extra-nodal extension and bone marrow involvement with consequent cytopenia (due to hemophagocytosis) obscure the prognosis.

The diagnosis of HS was based on histology and cytomorphology examinations, along with an immunohistochemical profile that pointed toward histiocytic lineage. Histologically, the lesion was composed of non-cohesive large cells with polygonal to cuboidal shape. These large neoplastic cells have abundant eosinophilic cytoplasm with vacuoles and a large, oval, eccentrically-located nucleus with vesicular chromatin and prominent, irregular nucleoli. Rarely, hemophagocytosis and giant cells may also be seen.

Cytomorphologic findings are not specific for HS; hence, immunohistochemical stains were utilized to prove histiocytic differentiation. The neoplastic cells were positive for CD68, CD163, CD14, CD4, CD11c, lysozyme, and alpha-1-antitrypsin and negative for epithelial and hematolymphoid differentiation markers. The proliferation index was highly variable, demonstrated by Ki-67 (range from 1 to 99%). Some genetic aberrations have been variably detected in cases of HS, among which BRAF (present in up to 70% of cases), *HRAS*, and *BRAF* gene fusion are the most frequently linked.

The conclusive diagnosis of HS is based on not only histological and immunohistochemical examination of histiocytic differentiation but also exclusion of other immunophenotypes, including lymphoid, epithelial, and melanocytic differentiation. Among the differential diagnoses, Langerhans cell histiocytosis (LCH), hemophagocytic lymphohistiocytosis, follicular cell sarcoma, and interdigitating cell sarcoma are among the most emphasized. HS and LCH are both determined by histiocytic proliferation. However, these entities have structural, enzymatic, and immunohistochemical differences that allow for proper differentiation in the majority of cases. The presence of positive immunohistochemistry for alpha-1-antitrypsin and lysozymes in this case effectively ruled out LCH. Follicular dendritic cell sarcoma was also discarded using the same criteria for LCH. Interdigitating dendritic cell sarcoma shares an immunohistochemical profile closely related to HS, while still being negative for alpha-1-antitrypsin. *BRAF* mutation has not been detected in these cases. The presence of positive staining for lysozymes can obscure the differential diagnosis; however, the ultrastructural examination demonstrated the scattered distribution of these instead of abundant and evenly distributed lysozymes in HS. Hemophagocytic lymphohistiocytosis is also a neoplastic proliferation of histiocytes by definition. Hematophagocytosis, while found in both entities, is more common and prominent in the former and constitutes a rare finding in the latter.

Imaging findings are essential for the early diagnosis of HS. However, satisfactory diagnostic imaging characteristics are still lacking. Contrast-enhanced CT scans demonstrated multiple, partially confluent, hypoattenuating masses in the enlarged spleen with multiple liver infiltration, and ultrasonography revealed multiple, ill-defined hypoechoic lesions. HS commonly presents as multiple hypointense T1 and hyperintense T2 lesions on MRI, which can also be found in cases of splenic neoplasm

Table 1 Comparison of main clinical features among the reported cases with primary splenic histiocytic sarcoma (including our case)

Case [Ref.]	Age (yr)/Sex	Chief complaint	Imaging	Weight of spleen	Treatment	Survival	Metastasis
1[6]	38/male	Weakness	Multinodular	264 g	S	-	-
2[2]	29/male	Edema	Nodular	735 g	S (R+C)	5 Yr 1 Mo	Liver
3[2]	60/male	Thrombocytopenia	Nodular	610 g	S+C	1 Yr 6 Mo	Liver, BM
4[2]	66/female	Edema, anemia	Multinodular	750 g	R+S+C	2 Yr 6 Mo	Liver, BM
5[15]	71/female	Thrombocytopenia	Multinodular	470 g	C	6 Mo	Hilum LN
6[16]	82/female	Evans syn	Multinodular	110 g	R	1 Mo	Liver
7[17]	58/female	Anemia	Nodular	800 g	R+S+C	3 Mo	-
8[18]	67/female	Thrombocytopenia	Non-nodular	760 g	S	6 Mo	BM
9[19]	81/female	Anemia, thrombocytopenia	Multinodular	236 g	S	5 Mo	Liver
10	40/female	Thrombocytopenia	Multinodular	110.1 g	S	-	BM

S: Surgery (splenectomy); R: Radiation; C: Chemotherapy; BM: Bone marrow; LN: Lymph node.

such as splenic lymphoma, hemagiomatosis, or angiosarcoma. Case reports have indicated the possible role of PET scan in histiocytic lineage disorders, such as LCH[12], and PET scans can be very valuable in the evaluation of disease dissemination and tailoring treatment in HS[13]. In our patient, initially, among those multiple splenic lesions on MRI, only one of the lesions along the splenic hilum showed a mild SUV of 3.5 on PET/CT, and all other splenic nodules showed non-avidity on the PET scan. No significant interval avidity changes were seen at previous active bone sites in the repeated PET scan three months later. However, progression of bone marrow metastasis was seen five months later, which is consistent with the characteristics of low-grade disease.

Splenectomy is useful for definitive diagnosis of the disease. At the early stage of the disease, splenectomy would be expected to be beneficial for those with HS of the spleen to prevent continuous dissemination from the primary tumor site. Our patient presented with multiple splenic lesions with a disseminated bone lesion. Two bone marrow biopsies and FNA of lymph nodes were inconclusive concerning the diagnosis. Moreover, our patient suffered from progressive thrombocytopenia. Laparoscopic splenectomy was performed for both therapeutic and diagnostic purposes. There is no standard treatment regimen for patients with HS, and patients should be encouraged to enroll in a clinical trial if one is available[14]. Adjuvant treatments of HS include radiotherapy, chemotherapy, and combinations thereof, depending on the stage of the disease. Lymphoma-type systemic chemotherapy has been recommended for multiple systemic diseases. Nine previously reported cases of HS of the spleen recovered asymptomatically after splenectomy for a definitive diagnosis (including five patients who underwent subsequent chemotherapy[2]). All but one patient (who was alive 13 mo after splenectomy and whose outcome is not yet known) died from liver infiltration[6,15].

Our patient presented with disseminated bone metastasis and multiple splenic lesions, without initial splenomegaly. Her bone marrow metastases were stable and then progressed at eight months after diagnosis without adjuvant treatment. She remains in good performance status without other distant organ metastases. Her low-grade differentiated tumor may be associated with a slow progressive disease.

CONCLUSION

HS of the spleen is a rare, lethal disease. The pathologic diagnosis can be difficult due to its differentiation with malignant LCH or other benign histiocytic proliferation, as in our case. PET scans can be very valuable in the evaluation of disease dissemination. Low-grade differentiation may be associated with a slow progressive disease.

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REFERENCES

1 **Grogan TM**, Pileri SA, Chan JKC, Weiss LM, Fletcher CDM. Histiocytic sarcoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: ARC Press, 2008; 356-357

2 **Kimura H**, Nasu K, Sakai C, Shiga Y, Miyamoto E, Shintaku M, Wakatsuki S, Tominaga K, Abe M, Maruyama Y. Histiocytic sarcoma of the spleen associated with hypoalbuminemia, hypo gamma-globulinemia and thrombocytopenia as a possibly unique clinical entity--report of three cases. *Leuk Lymphoma* 1998; **31**: 217-224 [PMID: 9720731 DOI: 10.3109/10428199809057601]

3 **Lauritzen AF**, Delsol G, Hansen NE, Horn T, Ersbøll J, Hou-Jensen K, Ralfkiaer E. Histiocytic sarcomas and monoblastic leukemias. A clinical, histologic, and immunophenotypical study. *Am J Clin Pathol* 1994; **102**: 45-54 [PMID: 8037167 DOI: 10.1093/ajcp/102.1.45]

4 **Risdall RJ**, McKenna RW, Nesbit ME, Krivit W, Balfour HH, Simmons RL, Brunning RD. Virus-associated hemophagocytic syndrome: a benign histiocytic proliferation distinct from malignant histiocytosis. *Cancer* 1979; **44**: 993-1002 [DOI: 10.1002/1097-0142(197909)44:3<993::AID-CNCR2820440329>3.0.CO;2-5]

5 **Cattoretti G**, Villa A, Vezzoni P, Giardini R, Lombardi L, Rilke F. Malignant histiocytosis. A phenotypic and genotypic investigation. *Am J Pathol* 1990; **136**: 1009-1019 [PMID: 2349962]

6 **Franchino C**, Reich C, Distenfeld A, Ubriaco A, Knowles DM. A clinicopathologically distinctive primary splenic histiocytic neoplasm. Demonstration of its histiocyte derivation by immunophenotypic and molecular genetic analysis. *Am J Surg Pathol* 1988; **12**: 398-404 [PMID: 3284398 DOI: 10.1097/00000478-198805000-00008]

7 **Jaffe E**. Histiocytic and dendritic cell neoplasms. In: Jaffe ESHN, Stein H, Vardiman JW, editors. World health organization classification of tumours: pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC, 2001; 273–277

8 **Copie-Bergman C**, Wotherspoon AC, Norton AJ, Diss TC, Isaacson PG. True histiocytic lymphoma: a morphologic, immunohistochemical, and molecular genetic study of 13 cases. *Am J Surg Pathol* 1998; **22**: 1386-1392 [PMID: 9808131 DOI: 10.1097/00000478-199811000-00009]

9 **Vos JA**, Abbondanzo SL, Barekman CL, Andriko JW, Miettinen M, Aguilera NS. Histiocytic sarcoma: a study of five cases including the histiocyte marker CD163. *Mod Pathol* 2005; **18**: 693-704 [PMID: 15696128 DOI: 10.1038/modpathol.3800346]

10 **Hornick JL**, Jaffe ES, Fletcher CD. Extranodal histiocytic sarcoma: clinicopathologic analysis of 14 cases of a rare epithelioid malignancy. *Am J Surg Pathol* 2004; **28**: 1133-1144 [PMID: 15316312 DOI: 10.1097/01.pas.0000131541.95394.23]

11 **Blum R**, Seymour JF, Hicks RJ. Role of 18FDG-positron emission tomography scanning in the management of histiocytosis. *Leuk Lymphoma* 2002; **43**: 2155-2157 [PMID: 12533041 DOI: 10.1080/1042819021000032908]

12 **Kaste SC**, Rodriguez-Galindo C, McCarville ME, Shulkin BL. PET-CT in pediatric Langerhans cell histiocytosis. *Pediatr Radiol* 2007; **37**: 615-622 [PMID: 17564738 DOI: 10.1007/s00247-007-0467-4]

13 **Yaman E**, Ozturk B, Erdem O, Gokcora N, Coskun U, Uluoglu O, Benekli M. Histiocytic sarcoma: PET-CT evaluation of a rare entity. *Ann Nucl Med* 2008; **22**: 715-717 [PMID: 18982475 DOI: 10.1007/s12149-008-0175-7]

14 **Jaobsen E**. Histioytic sarcoma. Available from: https://www.uptodate.com/contents/histiocytic-sarcoma

15 **Audouin J**, Vercelli-Retta J, Le Tourneau A, Adida C, Camilleri-Broët S, Molina T, Diebold J. Primary histiocytic sarcoma of the spleen associated with erythrophagocytic histiocytosis. *Pathol Res Pract* 2003; **199**: 107-112 [PMID: 12747473 DOI: 10.1078/0344-0338-00362]

16 **Kobayashi S**, Kimura F, Hama Y, Ogura K, Torikai H, Kobayashi A, Ikeda T, Sato K, Aida S, Kosuda S, Motoyoshi K. Histiocytic sarcoma of the spleen: case report of asymptomatic onset of thrombocytopenia and complex imaging features. *Int J Hematol* 2008; **87**: 83-87 [PMID: 18224419 DOI: 10.1007/s12185-007-0008-9]

17 **Oka K**, Nakamine H, Maeda K, Yamakawa M, Imai H, Tada K, Ito M, Watanabe Y, Suzuki H, Iwasa M, Tanaka I. Primary histiocytic sarcoma of the spleen associated with hemophagocytosis. *Int J Hematol* 2008; **87**: 405-409 [PMID: 18327679 DOI: 10.1007/s12185-008-0059-6]

18 **Yamada S**, Tasaki T, Satoh N, Nabeshima A, Kitada S, Noguchi H, Yamada K, Takeshita M, Sasaguri Y. Primary splenic histiocytic sarcoma complicated with prolonged idiopathic thrombocytopenia and secondary bone marrow involvement: a unique surgical case presenting with splenomegaly but non-nodular lesions. *Diagn Pathol* 2012; **7**: 143 [PMID: 23075171 DOI: 10.1186/1746-1596-7-143]

19 **Yamamoto S**, Tsukamoto T, Kanazawa A, Shimizu S, Morimura K, Toyokawa T, Xiang Z, Sakurai K, Fukuoka T, Yoshida K, Takii M, Inoue K. Laparoscopic splenectomy for histiocytic sarcoma of the spleen. *World J Gastrointest Surg* 2013; **5**: 129-134 [PMID: 23717746 DOI: 10.4240/wjgs.v5.i4.129]

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