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REVIEW

Embracing cancer immunotherapy with vital micronutrients

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Abstract

Immunotherapy is now commonly prescribed to cancer patients, but autoimmune-related adverse events are considerable. For severe, life-threatening side effects, cessation of therapy seems unavoidable, let alone intensive medical care required for patching up the adverse events. Even without serious adverse events, the response rates are too low and various combinatory regimens have been tried. However, toxicities are also added on, unless the adjuvant agents have remarkably few side effects. Actually, micronutrients are usually taken by a majority of cancer patients as nutritional support or to boost the immune function, let alone hoping to counteract treatment side effects. Recent studies have shown that combinations of micronutrients exert pleiotropic effects in controlling tumor growth and metastasis by modulating the tumor microenvironment, enhancing gut microbiota immune functions, and providing adjunct nutritional support to micronutrient deficient cancer patients. A higher than recommended dietary allowance micronutrient dose is proposed to reduce the toxic free radicals generated as a result of immunotherapy and tumor metabolism. This is not only helpful for managing treatment side effects but also enhancing treatment efficacy. As micronutrient supplementation is also useful to improve patients' quality of life, prolong survival, and sustain compliance to immunotherapy, further investigations are mandatory.

Key Words: Immunotherapy; Micronutrients; Immune-related adverse events; Vitamins; Tumor microenvironment; Immunonutrition

Core Tip: Micronutrients in combination may enhance immunotherapy efficacy by

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immunomodulation and minimizing immune-related adverse events, improve acquired immune response through modification of the tumor microenvironment, enhance gut-microbiota immune functions, boost immune-nutrition function, and improve patient outcome.

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INTRODUCTION

It was estimated that 30% to 90% of cancer patients took some form of supplements and micronutrients for immunity support and reducing treatment side effects upon being diagnosed with cancer. Micronutrients such as various vitamins and minerals, especially selenium, zinc, *etc.*, are often consumed without any discussion with their oncologists for fear of being criticized. After all, the role of micronutrients for cancer patients is not generally accepted. Actually, micronutrients such as vitamin C (usually at high dosages) have been used since its discovery in the 1930s not just as a nutritional supplement but also as an anti-microbial agent when there were no potent anti-microbial agents by then[1,2]. Currently, micronutrients are much more often employed by naturopaths and complementary and integrative medical practitioners with or without other modalities to treat chronic diseases, autoimmune disorders, and even cancers[3]. Even in this era of cancer immunotherapy, various immune-related adverse events (irAEs) constitute a real concern. Nevertheless, micronutrients may well be useful for tackling some of these adverse events and even enhance the efficacy, as is being alluded to in this review.

CANCER IMMUNOTHERAPY: IRAES

Checkpoint protein inhibitors (CPIs), including cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) inhibitors and programmed cell death protein 1 pathway/programmed cell death protein 1 ligand (PD-1/PDL-1) inhibitors, are now commonly employed to treat a progressively wider spectrum of cancers with fewer side effects and much better tolerance than classical chemotherapy[4]. Unfortunately, the response rates are low and the immune-related toxicities are considerable[5]. CPIs act by enhancing the immune function of T cells by blocking the connection between PD-1 and PDL-1 and preventing the inhibition of T cells. T cell cytotoxicity then attacks the tumor cells. CTLA-4 blocks the connection between dendritic cells and T cells related to CTLA-4. CTLA-4 removes the inhibition related to dendritic cells on T cells to achieve a cancer-killing effect. Because checkpoints may also regulate autoreactivity, immune checkpoint inhibitor therapy is complicated by irAEs[6]. The mechanisms leading to irAEs are similar to those promoting anti-tumor responses, which involve T and B cell immune modulation and induce autoantibody production [7]. However, the wide range of irAEs associated with immune checkpoint blockade may be diverse and serious. These may well lead to the suspension of the otherwise effective immunotherapy. The irAEs may affect various organs and patients would have multiple side effects. In a study of 78 patients receiving CPIs, 53% developed irAEs with 15% of patients developing more than one complication[4]. Notably, a small number of side effects are life-threatening or require urgent medical attention [8]. Some serious irAEs are colitis, interstitial pneumonitis, myocarditis, pericarditis, arrhythmia, impaired ventricular function, and vasculitis. Neurological complications such as myasthenia gravis, Guillain-Barrie syndrome or peripheral neuropathy, aseptic meningitis, and encephalitis are also documented. Endocrine side effects such as hypothyroidism, hyperthyroidism, adrenal insufficiency, and type I diabetes mellitus, as well as hepatitis, nephritis, autoimmune hemolytic anemia, thrombocytopenia, skin rashes, and bullous dermatoses are also seen[9]. Since many of these side effects are related to similar immunologic actions for the immunotherapy therapeutic effects, the management of such adverse events constitutes a major challenge. Ideally, an efficient

adjuvant drug should be available to enhance cancer immunity whilst alleviating the irAEs[10]; otherwise, irAEs may preclude the continuation of CPIs[8,11]. Currently, medical management of irAEs may often be limited to symptomatic relief with systemic corticosteroids or immunosuppressants together with specialist care. There is a great need for multidisciplinary guidance from different specialties to establish broad-based perspectives in early recognition and management of organ-specific irAEs and to set up management guidelines[12]. Notably, the Society for Immunotherapy of Cancer has set up such a multidisciplinary Toxicity Management Working Group to develop recommendations and initiate treatment protocols for irAEs[11].

ROLE OF VITAL MICRONUTRIENTS IN IMMUNE FUNCTION AND INFECTION

Micronutrients such as vitamins A, D, C, E, B6, and B12, folate, zinc, iron, copper, and selenium are best tailored according to age-related needs[13]. As adequate amounts of these micronutrients are vital for proper immune functioning[14], a high enough dose is necessary for various kinds of immuno-compromised or even the terminally ill[15,16]. According to some studies, micronutrients with the strongest evidence for immune support are vitamin C, vitamin D, and zinc[15,17,18].

Patients with micronutrient deficiencies are prone to various infections and even body dysfunctions due to weakened immune responses to pathogens such as viruses like SARS-CoV-2, the virus that causes COVID-19[19]. Strikingly, micronutrient deficiencies affect about two billion people worldwide[20], contribute to low immunity against infections, and constitute a common cause of immunodeficiency in developing countries[21]. On the other hand, micronutrient supplementation could enhance immune functions and help the body to fight against pathogens and cancers[15,22-24].

CLINICAL IMPACT OF MICRONUTRITION IN CANCER TREATMENT

Since the 1980s, there was abundant epidemiologic evidence that high intakes of fruits and vegetables reduced the risks of most cancers. This may support the concept that micronutrients could play a vital role in cancer prevention[24]. Recent systematic reviews on micronutrients and breast cancer[25] have shown that micronutrient consumption may reduce the incidence rates and/or progression of cancers[24]. Epidemiological and experimental studies showed that the percentage of cancer-related deaths attributable to diet and tobacco was as high as 60%-70% worldwide[26]. For micronutrients, *in vitro* and *in vivo* studies on over 50 human cancer cell lines have demonstrated a good anti-cancer effect being achieved in combinations of micronutrients (rather than the individual compounds). It was also well documented that nutrient combinations exert pleiotropic effects in controlling tumor growth, invasion, and metastasis[16,27-29].

CONTROVERSY OVER USE OF MICRONUTRIENTS IN CANCER THERAPY

Since most micronutrients may also act as antioxidants, some physicians are concerned about possible inhibitory effects on chemotherapy killing actions[30]. On the contrary, there are reliable studies on the beneficial effects of antioxidants and micronutrients for patients during radiation therapy[31,32] and chemotherapy[33,34]. A recent extensive review comprising of 174 peer-reviewed articles and 93 clinical trials with a total of 18208 cancer patients showed that antioxidants have superior potentials in reducing chemotherapy-induced toxicity[35]. The conclusion was that antioxidant supplementation during oncology treatments enhanced chemotherapeutic efficacy and even prolonged patient survival. Moreover, in other studies, when antioxidants were given concurrently with chemotherapy, no interference occurred. Rather, they enhanced the chemotherapeutic effects, and even protected normal tissues and increased patient survivals and therapeutic responses[36,37].

VITAL MICRONUTRIENTS — ROLE IN AMELIORATING IRAES AND ENHANCING IMMUNOTHERAPY

Tumor microenvironment modification

The tumor microenvironment (TME) is largely composed of mesenchymal stem cells, fibroblasts, endothelial cells, adipocytes, and immune cells with an altered extracellular matrix having an acidic and hypoxic composition. TMEs can promote immune tolerance through the secretion of lactate and competing for nutrients between tumor cells and immune cells[38]. Cancer-associated fibroblasts and solid tumors can promote immunosuppression by inhibiting T cell functions and extracellular matrix remodeling[39]. Recent studies have suggested that nutrients available in the TME can influence immunotherapy response and cancer cell metabolic pathways[38,40]. Micronutrients like vitamin C can enhance immune cell functions by modifying the TME by hypoxia-inducible factors[41]. High-dose vitamin C modulates infiltration of the TME by immune cells and delays cancer cell growth in a T cell-dependent manner. Vitamin C enhances the proliferation and maturation of T cells and natural killer cells[42]. It also reduces the formation of neutrophil extracellular traps in the TME, which are related to irAEs due to checkpoint blockade[43]. The combination of high-dose vitamin C and immune checkpoint therapy may potentially enhance the efficacy of immunotherapy for cancer[44].

Vitamin D supplementation also suppresses tumor angiogenesis, progression, and metastasis *via* targeting components of the TME[45]. The active form of vitamin D, $1,25(OH)_2D_3$, regulates stromal cells including tumor-associated fibroblasts, tumor-derived endothelial cells, cancer stem cells, and infiltrating immune cells within the TME to facilitate cancer suppression. Vitamin D also has anti-inflammatory effects within the TME. This leads to the inhibition of proliferation, induction of apoptosis and differentiation, suppression of migration, and autophagic cell death of tumor cells [45]. Taken together, these may reaffirm the anti-cancer potential of vitamin D[46].

Enhancing gut microbiota immune functions

Micronutrient deficiencies have been linked to changes of bacterial species in the human gut microbiota affecting the host regulation of immune responses[47]. The activity of the gut microbiota has significantly contributed to the host immune health and is linked to the development of many diseases including cancer. Therapeutic interventions to optimize microbiota composition to improve immunotherapy outcomes have shown promising results[48,49]. In addition, gut microbiota modulations through micronutrient supplementations could effectively enhance efficacy and relieve or tackle resistance during immunotherapy treatments[50]. Gut microbiota may also activate or repress the host's response to CPIs and potentially modulate resistance to cancer immunotherapy[51]. As vitamin D deficiency has been linked to gut dysbiosis and bowel inflammation, vitamin D may play a significant role in gut microbiome regulation and host immune responses[52]. Moreover, vitamin D supplementation has been shown to increase gut microbial diversity significantly. This is a positive health impact on healthy individuals[53] and cancer patients[54].

Adjunct nutrition support for cancer patients

It was estimated that about 30%-90% of patients believed that they had inadequate diets leading to nutritional deficiencies and poor immune functions; some cancer patients were obviously cachexic. Micronutrient deficiencies do have negative impacts on immunotherapy as the host's immunocompetence is weakened. There is also an increased risk of developing irAEs and a negative impact on the patient's quality of life. Nutritional deficiencies can be reversed early if adjunct micronutrients are given before and during oncology treatments. Some chemotherapy drugs may have side effects of depleting certain micronutrients. This tends to worsen the nutritional deficiency, *e.g.*, cyclophosphamide and paclitaxel can deplete vitamin D by an increased breakdown of calcidiol and calcitriol[55]. A cohort study from the Mayo Clinic has shown a 26% reduction of non-small cell lung cancer mortality with improved quality of life and prolonged survival through micronutrient supplementation[56]. Apparently, immunonutrition has the potential to modulate the activity of the immune system by interventions with specific nutrients. It may be applied with immunotherapy to improve immune functions, modulate the acquired immune response, decrease treatment toxicity, and enhance patient outcomes[57]. Micronutrients such as selenium, vitamin C, and vitamin D (at high doses) have been found to be effective and safe for patients undergoing oncological intervention[16,55,58,59].

Protecting normal healthy cells

Immunotherapy-associated irAEs include autoimmune reactions, cytokine release syndromes, and vascular leak syndrome. These vary depending on the type of immunotherapy and the specific mechanism of action. Cytokines such as high-dose IL-2 will lead to capillary leakage and a sepsis-like syndrome or multi-organ failure[60]. CPIs disinhibiting T cell anti-tumor action can lead to a distinct constellation of organ-specific inflammatory side effects or irAEs[12].

Vitamin D and zinc have been known for balancing immune functions through the prevention and treatment of autoimmune diseases[61]. Several observational studies have shown that vitamin D deficiencies increased the risk of autoimmune diseases such as type I diabetes, systemic lupus erythematosus, inflammatory bowel disease, Hashimoto's thyroiditis, multiple sclerosis, psoriasis, and rheumatoid arthritis[62,63]. Vitamin D supplementation is found to be beneficial to prostate, breast, and colorectal cancers and melanoma patients during treatment[64].

Vitamin B12 supplements may reduce the direct toxic side effects of immunotherapy as vitamin B12 is required for red blood cell synthesis, neural functions, and reduction of the severity of drug-induced peripheral neuropathy[65]. Vitamin B12 has been added as a supplement to pemetrexed and cisplatin chemotherapy agents, as used in pleural mesothelioma and non-small cell lung cancer. This was allegedly because of its folate similarity and inhibition of purine and pyrimidine synthesis[66]. Vitamin B12 effectively reduced the toxic side effects of the main chemotherapy.

Vitamin C is concentrated in most immune cells which support essential immune functions such as enzyme cofactors for Fe- or Cu- containing oxygenase. This regulates cell metabolism, epigenetics, growth, survival pathways, and even stem cell phenotypes[42]. High-dose intravenous vitamin C has been found to be useful as an adjunct to interleukin-2 immunotherapy to reduce capillary leakage, systemic complement activation, and a non-specific rise in inflammatory mediators such as TNF-alpha and C-reactive proteins by protecting the endothelium from inflammation [67]. High-dose intravenous vitamin C may also reduce cytokines which cause tumor angiogenesis and inflammation in cancer patients[68].

Vitamin D deficiency has been linked to autoimmune diseases[63] such as psoriasis, vitiligo[69,70], autoimmune thyroid diseases, Hashimoto's thyroiditis, and postpartum thyroiditis[71]. Vitamin D decreases the expression of various cytokines that cause vitiligo and other autoimmune disorders by preventing the destruction of melanocytes [69]. Oral vitamin D3 has been reported to be effective for improving the levels of epidermal keratin in psoriatic patients and to improve the treatment outcome with topical dithranol, PUVA (psoralen and ultraviolet A, a light therapy for skin diseases), and oral etretinate and hydroxyurea therapy[72]. A pilot study with prolonged supplementation of high dose vitamin D has improved the clinical course of vitiligo and psoriasis[73]. Melanoma patients often present with cutaneous lesions such as vitiligo, representing an autoimmune disorder with progressive destruction of melanocytes[74]. Dermatologic side effects such as vitiligo and leukoderma are often seen in melanoma patients who are on PD-1 inhibitors (up to 10%, more for ipilimumab)[75]. Notably, irAEs affect all organ systems and most commonly the skin (pruritus, rash, and vitiligo), the gastrointestinal tract (enterocolitis), the liver (hepatitis), and the endocrine system while less commonly involve the neurological system. The gastrointestinal tract, liver, lung, and skin are actually maintained in an immunologically quiescent state, which may explain the vulnerability of these organs for the development of irAEs[6].

MICRONUTRIENTS: VENTURING TO REDUCE AUTOIMMUNE-RELATED IRAES

Interestingly, a recent cohort study has shown that vitamin D supplementation could reduce the risks of CPI-induced colitis by as much as 65%[76]. As CPI-induced colitis is an irAE that is basically autoimmune-related, such micronutrients as vitamin D may also reduce the risks of other CPI-induced and autoimmune-related irAEs. As alluded to above, vitamin D deficiency is rather closely linked with autoimmune disorders, let alone vitamin D administration may be beneficial. Hence, it would appear highly worthwhile to look at the prospects of such micronutrients in managing autoimmune-related disorders. There may be a potential role of micronutrients in preventing irAEs induced by CPIs. Currently, CPIs do have considerable autoimmune-related irAEs. For instance, the phase 2 KEYNOTE-224 trial of pembrolizumab for advanced hepatocellular carcinoma patients who have been treated previously with sorafenib saw

considerable adverse events[77]. In that trial, treatment-related adverse events occurred in 73% of 104 patients. Most of the more serious adverse events were immune-related. Naturally, serious adverse events may well lead to dropouts or suspension of the immunotherapy, defeating the whole purpose of such a valuable modality of treatment. Apparently, it would be worthwhile to examine whether vitamin D or zinc really has beneficial effects on the management of autoimmune disorders. If so, it may support the feasibility of using these micronutrients prospectively to reduce the autoimmune-related irAEs of CPIs. If some simple measures could prevent or reduce such adverse events, it would be most helpful. More cancer patients may then be able to benefit from CPIs. Before that could ever happen, one could start by scrutinizing how effective are these micronutrients, especially vitamin D and zinc for the management of autoimmune-related disorders. Table 1[78-85] shows selected trials of zinc and vitamin D on autoimmune-related disorders.

Notably, the 3rd study listed in Table 1 involved a combination of zinc and vitamin A supplementation that had been shown to improve serum apoprotein A-1 and apoprotein B levels and the apoprotein B/proprotein A-1 ratio in patients with type 1 diabetes mellitus (T1DM). In fact, the deficiency of vitamin A would mainly involve an impaired transport mechanism of vitamin A from its hepatic storage to the target sites [86]. As insulin therapy would reverse this impairment, the replacement of vitamin A may not be crucial for controlling T1DM. Hence, the beneficial adjuvant effect of the combination of zinc and vitamin A for T1DM was more likely to be due to zinc than vitamin A. Moreover, from Table 1, three studies had involved T1DM cases of recent onset (studies 4, 5, and 6). Apparently, the adjuvant role of micronutrients for T1DM cases of recent onset may be more effective. Possibly, the fact that a vitamin D analog could benefit recent-onset T1DM may suggest that it would be useful to prevent an irAE that involves the beta cells of the pancreas.

Moreover, as micronutrients are but adjunctive treatment modalities, for demonstrating their effectiveness would also depend largely on the main modalities of treatment. In case that there is a significant difference in the effectiveness of those main modalities of treatment between the study groups, then the effectiveness of the adjunctive modalities of treatment would be difficult to demonstrate. Another highly relevant factor is the distribution of genetic predispositions between various groups of the study population. As to balance very evenly the genetic predispositions among the groups is not done easily or not done at all, the effect of such an imbalance between the groups would naturally affect the results[87]. Thus, incidental negative trial findings of micronutrients should not be taken as definitive proof that micronutrients are not useful.

Lastly, even the diet may affect autoimmunity. It was reported that heavy metals like mercury[88] might be incriminated. Chronic exposure to low levels of methylmercury (organic) and inorganic mercury was common among 1352 female subjects 16 to 49 years of age from the US National Health and Nutrition Examination Survey. Probably, the mercury was from consuming fish and even the slow disintegration of dental amalgams. Also, 16% of subjects were antinuclear antibody (ANA) positive. Hair and blood mercury levels were associated with ANA positivity. As ANA is closely related to autoimmune disorders, methylmercury exposure was deemed to be associated with subclinical autoimmunity among subjects and autoantibodies may even predate the onset of clinical diseases by years.

Taken together, several factors may affect the effectiveness of vitamin D and zinc on autoimmune disorders. When trials were performed on such micronutrients, it was challenging to balance evenly all the relevant factors among different arms of those studies. As such, results can be rather variable but may not reflect the true effectiveness of these micronutrients. Thus, negative clinical trial results should not be taken at their face value. After all, all these adjuvants have to act together with other more specific agents before exerting their effects. Moreover, the duration of onset of the autoimmune-related disorders may also be highly relevant. It is also possible that such adjuvant agents are most effective for prevention rather than treatment. In any case, these micronutrients should be further investigated thoroughly for their ability of preventing or reducing early autoimmune-related irAEs induced by CPIs. This is especially so as they have an excellent safety profile, are easily taken and eminently affordable.

Actually, cancer patients who are also suffering concurrently from immune disorders are routinely precluded from receiving any CPI, even if they are already on specific drugs for their autoimmune disorders. This is because of the fear of exacerbating their autoimmune symptoms once CPIs commence. If more studies can be done on vitamin D and zinc on their ability to prevent exacerbation of autoimmune disorder symptoms, one may know how effective these can prevent such autoimmune-related

Table 1 Selected trials on the effect of zinc and vitamin D on autoimmune related disorders

No.	Autoimmune disorder	Agent	Dose	Period	Trial type	Benefit	Year
1	MS	Cholecalciferol	50000 IU/wk	12 mo	R, C, DB	Decreased incidence rate of demyelination plaques, reduced progression risk	2013[78]
2	RA	ZnSO4	220 mg/3×/d	12 wk + 12 wk	C then O	Decreased joint swelling, stiffness, walking time	1976[79]
3	T1DM	ZnSO4 + vit A	10 mg/d + vit A 25000 IU	12 wk	R, C, DB	Increased serum apo A1; decreased apo B/Apo A1 ratio	2010[80]
4	T1DM (RO)	Alpha-calcidol	10 IU/1-2×/d	6 mo	R, C, B (prtps)	FCP higher; lower requirement of insulin	2013[81]
5	T1DM (RO)	Cholecalciferol	2000 IU/d	18 mo	R, C, DB	Protective immunologic effect; slow decline of residual β-cell function (serum FCP and SCP levels)	2012[82]
6	T1DM (RO)	Cholecalciferol	70 IU/kg/d	12 mo	R, C, DB	Improved the suppressive capacity of Tregs	2015[83]
7	PS	Zinc pyrithione topical 0.25% in an emollient base	2×/d	3 mo	R, C, DB	Decreased plaques/PASI score	2011[84]
8	SLE	Vit D	50000 IU/wk	24 wk	R, C, DB	Decreased disease activity parameters; reduced fatigue	2016[85]

Apo: Apoprotein; B: Blind; C: Controlled; DB: Double blind; FCP: Fasting C-peptide; MS: Multiple sclerosis; O: Open; PASI: Psoriasis area and severity index; prtps: Participants; PS: Psoriasis; R: Randomized; RA: Rheumatoid arthritis; RO: Recent onset; SCP: Stimulated C-peptide; SLE: Systemic lupus erythematosus; T1DM: Type 1 diabetes mellitus; Treg: Regulatory T cells; Vit: Vitamin.

irAEs of CPIs. Hopefully, these unfortunate cancer patients suffering from two major disorders may then benefit from CPIs. Even those patients without any pre-existing autoimmune disorders may also benefit from reduced autoimmune-related irAEs upon commencing CPIs. Their autoimmune-related irAEs may be reduced by micronutrients and those unplanned suspensions of CPIs are avoided. Even for those who already have such unfortunate suspensions, such micronutrients might still contribute to a more successful rechallenging program. After all, if there are no other realistic options than CPIs, the threat to life is actually higher for uncontrollable cancers than autoimmune-related irAEs.

Immunomodulating micronutrients enhances immunotherapy

Vitamin A, beta-carotene, folic acid, vitamin B12, vitamin C, vitamin D, riboflavin, iron, zinc, and selenium may all have immunomodulating functions and could enhance the immune response rates of immunotherapy and even reduce irAEs[89]. They play an important role in reducing oxidative stress in diseases and cancers. Vitamin A supplementation improves levels of IgA immunoglobulin and CD40 ligand-activated IgG and reduces inflammatory cytokine levels[90]. Vitamin E as a potent antioxidant would reduce inflammation by modulating T cell function and downmodulating prostaglandin E2 in patients[91]. Vitamin C improves immune functions by supporting natural killer cell activities, lymphocyte proliferation, and chemotaxis, stimulates dendritic cells to secrete interleukin-12, and activates T and B cell functions[42]. High-dose vitamin C not only enhances the cytotoxic activity of CD8 T cells but also enhances immunotherapy by co-operating with immune checkpoint therapy in several cancer types[44]. Vitamin B12 deficiency has been linked to low lymphocyte counts, impaired NK cell function, decreased CD8+ cells, and impaired immune functions. Eventually, the raised CD4/CD8 ratio[92] would be potentially reversible by oral or intramuscular B12 injections. Vitamin D [1,25-$(OH)_2D_3$] binds to the vitamin D receptor of both the antigen-presenting cells (APC), dendritic cells, and T lymphocytes so as to exert its indirect and direct effects on T lymphocytes. The latter effect on the T lymphocytes is a change towards a more tolerogenic (capable of producing immunological tolerance) state with induction of T helper-2 (Th2)-lymphocytes and regulatory T lymphocytes (Tregs), together with a downregulation of the pro-inflammatory Thelper-1 (Th-1)-lymphocytes, Thelper-17 (Th-17)-lymphocytes, and Thelper-9 (Th9)-lymphocytes][93]. Notably, vitamin D suppresses T cell prolif-

eration and then results in a shift from a Th-1 to a Th-2 development, inhibition of Th-17 cell development, and also facilitation of T regulatory cells with an arrest of cytotoxic T lymphocyte infiltration as well as increased $CD4^+CD25^+$ Tregs[94]. Lastly, vitamin D inhibits inflammatory cytokine production by monocytes, and suppresses dendritic cell differentiation and maturation. This helps to maintain tolerance and would also promote protective immunity[95].

DISCUSSION

Micronutrients are closely associated with the body's immune functions; a micronutrient deficient subject will have poor immune status and be prone to infections and even cancer development. Immunotherapy is emerging as an important adjunct oncology modality of treatment. The key to success is dependent on a good host's immune response to tackle cancers. The target of immunotherapy is killing the cancer cells with minimal collateral damages and leaving the body's immune system intact. Even though cancer immunotherapy provides a better option than chemotherapy, achieves higher success rates, and causes less marrow depression, it has considerable limitations. More than half of treated patients develop irAEs[4], let alone only a minority of cancer patients respond well to immunotherapy. Moreover, a minority of irAEs can be serious and even fatal. To overcome these limitations, supplementation of vital micronutrients to immunotherapy patients seems to be the simplest and the most pragmatic way of reducing such irAEs. Micronutrients have been used successfully in conventional oncology to reduce treatment side effects, enhance therapy efficacy, prolong survival, and improve quality of life[25,27,28,59,96]. For immunotherapy, despite less clinical experience, similar biophysiological mechanisms may also work when micronutrients are added to immunotherapy. Realistically, micronutrients may well offer comparable benefits to immunotherapy patients by strengthening the immune cell functions, enhancing tumor-killing effects, and reducing or preventing treatment complications[55].

Notably, micronutrient deficiency in one particular nutrient is rather difficult to diagnose and clinical symptoms may not be obvious, let alone overlapping effects with other clinical conditions. Thus, for best results, micronutrients as an adjunct oncology therapy should be given prospectively and in combination with the main treatment[15,97].

Unfortunately, there are no standard micronutrient supplementation protocols for immunotherapy patients. Despite some negative findings[37,98], a general consensus could still be built on the effectiveness of known positive trials and the remarkable safety profile of micronutrient therapy. After all, negative trials may well be due to various related factors and the imbalance of trial participants in various arms, as has been discussed in great detail. Moreover, as the antioxidant effect of micronutrients has already been proven to be not a concern, some studies advocate using higher than the recommended dietary allowance doses of micronutrients in combination for cancer patients to achieve optimal benefits[44,59,96,99]. A higher dose of micronutrients offering greater antioxidant effects may better tackle free radicals generated during immunotherapy and also enhance host immune function[15,100]. Importantly, future oncology research should be directed towards investigating the effects of different groups of micronutrients in combination with the main oncology modalities of treatment for different cancer types so as to delineate the optimal micronutrient regimens for immunotherapy.

CONCLUSION

Micronutrients used to play an active role in the past. High-dose vitamin C has been administered for viral infections before the debut of more specific agents; vitamin D has also been used for treating some autoimmune disorders before more specific agents are now available for such disorders. Currently, these and similar micronutrients should be investigated actively to better define their definitive adjuvant role in the era of cancer immunotherapy. Actually, micronutrients play a pivotal role in maintaining good immune cell functions and would also play an integral role in the defense against infectious agents and even cancers. Adequate amounts of micronutrients during immunotherapy have been shown to have the potential of enhancing immunotherapy efficacy, reducing irAEs, improving patients' quality of life, prolonging survivals, and even sustaining the best treatment compliance. As the use of

micronutrients as adjuvants for oncology treatments is still in its infancy, many more studies are required to explore the full potential of such safe, convenient, and affordable agents.

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REVIEW

Metastatic disease to the liver: Locoregional therapy strategies and outcomes

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Abstract

Secondary cancers of the liver are more than twenty times more common than primary tumors and are incurable in most cases. While surgical resection and systemic chemotherapy are often the first-line therapy for metastatic liver disease, a majority of patients present with bilobar disease not amenable to curative local resection. Furthermore, by the time metastasis to the liver has developed, many tumors demonstrate a degree of resistance to systemic chemotherapy. Fortunately, catheter-directed and percutaneous locoregional approaches have evolved as major treatment modalities for unresectable metastatic disease. These novel techniques can be used for diverse applications ranging from curative intent for small localized tumors, downstaging of large tumors for resection, or locoregional control and palliation of advanced disease. Their use has been associated with increased tumor response, increased disease-free and overall survival, and decreased morbidity and mortality in a broad range of metastatic disease. This review explores recent advances in liver-directed therapies for metastatic liver disease from primary colorectal, neuroendocrine, breast, and lung cancer, as well as uveal melanoma, cholangiocarcinoma, and sarcoma. Therapies discussed include bland transarterial embolization, chemoembolization, radioembolization, and ablative therapies, with a focus on current treatment approaches, outcomes of locoregional therapy, and future directions in each type of metastatic disease.

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Core Tip: Locoregional percutaneous catheter-directed approaches have been associated with better tumor response, improved disease-free and overall survival, and decreased morbidity in metastatic disease to the liver compared to standard treatment. This review explores recent advances in liver-directed therapies for metastatic liver disease from primary colorectal, neuroendocrine, breast, and lung cancer, as well as uveal melanoma, cholangiocarcinoma, and sarcoma. Therapies discussed include bland transarterial embolization, chemoembolization, radioembolization, and ablative therapies, with a focus on current treatment approaches, outcomes of locoregional therapy, and future directions in each type of metastatic disease.

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INTRODUCTION

Metastatic disease to the liver is the most common malignant liver condition and a major cause of cancer-related morbidity and mortality[1]. While colon cancer represents the most common metastatic disease to the liver, other common primary tumors include lung and breast adenocarcinomas, neuroendocrine tumors, melanomas, and sarcoma[2]. Regardless of primary tumor type, liver metastasis generally represents advanced disease, and is typically associated with poor prognosis[3]. The goals of therapy at this stage are often palliative, but there is a growing interest in differentiating between oligometastatic disease characterized by limited metastasis from more widespread metastatic disease, as these classifications may carry prognostic value[4-7]. In some cases, curative treatment has been demonstrated in oligometastatic disease, encouraging aggressive local treatment in appropriate patients[8]. Traditional management options for patients with metastatic disease to the liver include surgical resection and systemic chemotherapy. However, the percent of patients who present with disease amenable to surgery ranges from 25% to less than 10% depending on the primary tumor[9,10]. While significant advances in complex liver surgery have been made over the past several decades, liver resections are nevertheless still associated with major morbidity and mortality[11]. These risks must be carefully balanced with the evidence for a survival benefit especially in the setting of metastatic disease[12].

On the other hand, most patients with liver metastases have unresectable disease, either because of anatomical limitations, presence of extrahepatic disease, or absence of evidence establishing a survival benefit for resection. Fortunately, novel liver-directed strategies are being used to downstage tumors for curative resection, reduce symptoms, and provide better tumor control[13-15]. In the last ten years, locoregional therapies in metastatic liver disease have demonstrated comparable outcomes with fewer side effects than current standards of care, leading to formal incorporation into treatment algorithms as first-line, adjunctive, or second-line therapy for various tumor types[16-19]. The development of new image-guided techniques and enhanced targeted pharmaco- and radiotherapeutics promise to improve upon the impressive tumor response, progression free survival (PFS), and overall survival (OS) rates that these therapies have already demonstrated. This review examines the recent advances in locoregional therapy for metastatic disease to the liver including transarterial embolization (TAE), transarterial chemoembolization (TACE), transarterial radioembolization (TARE), and ablative therapies in major cancer types.

LOCOREGIONAL THERAPIES

TAE, TACE, and TARE

Catheter-directed locoregional therapies are based on the principle that liver tumors recruit their blood supply from the hepatic artery, while hepatic parenchymal cells are primarily supplied by the portal vein. In this way, local therapies such as TAE, TACE, and TARE can be targeted to tumor cells while minimizing damage to normal liver tissue[20]. In all cases, the key branches of the hepatic artery supplying the tumor are identified before the introduction of embolic agents, chemotherapy, or radiotherapy to prevent non-target embolization[21] (Tables 1 and 2).

In TAE, particulate or liquid embolic agents are administered, resulting in cellular membrane disruption and ischemic cell death. Similarly, in TACE, tumor vessels are occluded, with the added benefit of local delivery of chemotherapeutic agents. In the conventional approach (c-TACE), a lipiodolized chemotherapeutic is introduced, followed by the embolic agent. More recently, drug-eluting beads (DEB-TACE) have been used as both chemotherapeutic and embolic agents allowing for the sustained release of chemotherapy with greater standardization compared to c-TACE. The most common complication of these procedures is postembolization syndrome (PES), which presents as self-limiting right upper quadrant pain, nausea, fever, and elevated liver function tests. PES is attributed to tumor necrosis and tissue ischemia and full recovery within seven to ten days is typical. Other risks include hepatic decompensation, renal injury, biliary injury, infection, and non-target embolization[21].

In a similar fashion, TARE uses 30-micron beads that have been embedded or coated with a radioisotope of yttrium (*i.e.*, ^{90}Y). Once introduced, ^{90}Y undergoes beta-decay causing radiation-induced damage to cellular DNA repair mechanisms and ultimately cell death. One benefit of TARE over TACE is that is can be delivered in the outpatient setting[22]. Unique complications of TARE include radioembolization-induced liver disease (REILD) and post-radiation syndrome. REILD is seen in up to 20% of patients treated with TARE and defined by jaundice and ascites that persist 1-2 mo after treatment without evidence of obstruction or tumor progression. In contrast, post-radiation syndrome is a set of non-specific symptoms including fatigue, nausea, anorexia, and fever generally requiring supportive management[23].

To assess response, follow up imaging and laboratory investigations are conducted 4-6 wk later, and every 3-6 mo thereafter to evaluate treatment success and monitor disease progression. Laboratory evaluation includes tumor markers such as CEA and CA19-9 for colorectal and cholangiocarcinoma[24] or chromogranin, pancreatic polypeptide, or pancreastatin for neuroendocrine tumors[25,26]. To evaluate response with imaging, multiple criteria have been created. One way to evaluate response is by assessing changes in tumor size with contrast-enhanced CT or MRI imaging, which is the basis of one commonly used set of response criteria, termed Response Evaluation Criteria in Solid Tumors (RECIST)[27]. Similarly, PET Response Criteria in Solid Tumors (PERCIST), was developed to measure changes in radiotracer uptake on positron emission topography imaging[28]. The development of novel therapies has led to the development of tumor-specific imaging criteria including the modified RECIST (mRECIST) criteria for hepatocellular carcinoma, Modified CT Response Evaluation (Choi) Criteria for gastrointestinal stromal tumors, and the European Association for Study of the Liver (EASL) criteria, among others[28-31]. These criteria were developed to take into account functional changes seen on imaging, such as contrast enhancement or density, when using therapies that may not lead to radiographic reductions in tumor size[32].

Ablative strategies

Ablative techniques include radiofrequency ablation (RFA), microwave ablation (MWA), cryoablation (CA), irreversible electroporation (IRE), laser-induced interstitial thermotherapy (LITT), and high-intensity focused ultrasound (HIFU). Commonly used ablative techniques can be performed *via* intravascular approach, percutaneously, or in conjunction with surgical resection. While RFA is generally the most commonly studied method, alternate techniques such as MWA have become increasingly popular. In contrast, the use of CA has declined due to its increased post-procedure morbidity and local recurrence rates compared with other methods[33,34]. CA is associated with a number of other unique adverse effects including myohemoglobinuria leading to acute renal failure, cardiac dysrhythmias, and cryogenic shock, a cytokine-mediated syndrome of multi-organ failure, severe coagulopathy, and disseminated intravascular coagulation[21,35]. IRE, LITT, and HIFU remain less well-studied modalities for treatment of liver metastases but have demonstrated promise in clinical

Table 1 Summary of locoregional therapy options for metastatic disease to the liver

Modality	Techniques	Risks
TAE	Particulate or liquid embolic agents	PES, liver abscess, liver biloma, liver failure
TACE	Conventional emulsified chemotherapeutic agent (c-TACE) or drug-eluting beads (DEB-TACE)	PES, liver abscess, liver biloma, liver failure
TARE	Yttrium-90 radioisotope loaded on microspheres	REILD, PRS, liver failure, liver abscess, liver biloma
Ablation	Radiofrequency, microwaves, laser, cooling, alternating and direct current	PAS, bleeding, damage to surrounding structures

PES: Post-embolization syndrome; REILD: Radioembolization-induced liver disease; PRS: Post-radioembolization syndrome; PAS: Post-ablation syndrome; TAE: Transarterial embolization; TACE: Transarterial chemoembolization; TARE: Transarterial radioembolization.

Table 2 Applications and outcomes of locoregional therapies by tumor type

Modality	Applications and outcomes
TAE	First-line for unresectable symptomatic well-differentiated NELM refractory to medical therapy [19]
	Improved OS and PFS *vs* first-line chemotherapy in unresectable CRLM[39,40]
TACE	Comparable tumor response and OS *vs* first-line chemotherapy in neoadjuvant setting for CRLM [41]
	Improved OS and tumor control when used as adjunctive therapy in BCLM[42-44]
	Comparable overall survival to systemic chemotherapy in UMLM[45,46]
	In IHC, DEB-TACE and chemotherapy have comparable OS[47] and DEB-TACE improves OS when added to chemotherapy[48,49]
	TARE with first-line chemotherapy offers a survival benefit in CRLM[50], IHC[51]
TARE	Provides survival benefit in CRLM after failure of two lines of chemotherapy[52]
	TARE plus chemotherapy improves downstaging *vs* chemotherapy alone in CRLM[13,53], IHC[51]
	Increases OS in unresectable CRLM compared to chemotherapy alone[54]
Ablation	RFA[55] and MWA[56] have comparable OS to surgical resection in CRLM
	RFA with resection has comparable OS to two-stage hepatectomy in CRLM[57], NELM[58]
	Fewer adverse events, longer PFS, and comparable OS *vs* resection in BCLM[12,59]

NELM: Neuroendocrine cancer with liver metastasis, CRLM: Colorectal cancer with liver metastasis, BCLM: Breast cancer with liver metastasis, UMLM: Uveal melanoma with liver metastasis, IHC: intrahepatic cholangiocarcinoma, OS: Overall survival, PFS: Progression-free survival; TAE: Transarterial embolization; TACE: Transarterial chemoembolization; TARE: Transarterial radioembolization.

studies[21] (Tables 1 and 2).

RFA uses a locally introduced electrode to emit radiofrequency alternating current to generate thermal energy that results in tumor necrosis. It is most effective in small tumors (< 3 cm) and in metastases with fewer lesions and is less effective in tumors located close to the hilum and large blood vessel due to the heat sink effect of flowing blood[36,37]. To combat size limitations, multiprobe stereotactic RFA is a technique that shows promise for hepatic tumors up to 8 cm[38]. MWA similarly uses a locally introduced antenna to generate an electromagnetic field that aligns nearby water molecules, producing thermal energy. In contrast to RFA, MWA achieves target temperatures faster over a larger area, produces more uniform heating zones, and is less susceptible to heat sink effects. Further, MWA has the ability to perform multiple ablations simultaneously[39]. The newest form of ablation is irreversible electroporation (IRE). In contrast to thermal ablation techniques, IRE uses high-voltage electrical current to create permanent nanopores in the cell membrane, leading to apoptosis[40]. Serious complications common to ablative strategies include bleeding, damage to surrounding organs such as the diaphragm, GI tract, and gall bladder, and a self-limiting post-ablation syndrome (PAS) that presents with the same symptoms as

PES[41,42].

COLORECTAL CARCINOMA

Introduction to colorectal liver metastasis

Colorectal liver metastasis (CRLM) is the most common type of liver malignancy, and over one half of patients with colorectal cancer will develop metastasis to the liver[43]. Interestingly, in left-sided colorectal cancer, liver metastasis is less extensive with better overall survival. In contrast, metastasis to the liver in right-sided colorectal cancer is more extensive with worse survival[44]. Surgical resection remains the first-line treatment for CRLM, but only about 25% of patients are surgical candidates[9]. In recent years, there has been a substantial increase in evidence supporting local therapies for surgically untreatable CRLM. Current guidelines now support the use of local therapies after neoadjuvant systemic chemotherapy has failed to successfully downgrade a surgically unresectable tumor[45]. Currently, ablation is being explored as an alternative to surgical management in select cases of CRLM. Additionally, the benefits of TARE and TACE in conjunction with systemic chemotherapy and ablation are being actively explored.

TACE in CRLM

While c-TACE with doxorubicin is commonly employed for primary hepatocellular carcinoma, doxorubicin does not have the same efficacy against colorectal metastasis. In response, there has been growing interest in DEB-TACE with irinotecan (DEBIRI), a chemotherapeutic used primarily in the treatment of colorectal carcinoma. In 2012, Fiorentini *et al*[46] conducted the first randomized clinical trial on DEBIRI *vs* FOLFIRI (systemic irinotecan, fluorouracil, and leucovorin), which demonstrated the superiority of DEBIRI in terms of overall survival (22 *vs* 15 mo) and progression free survival (7 mo *vs* 4 mo). Metanalysis of studies since then have demonstrated an average tumor response rate of 62%, with median OS of 18 mo with DEBIRI, and 33 mo when DEBIRI is combined with FOLFOX[47]. DEBIRI has additionally been explored as a neoadjuvant therapy: PARAGON II demonstrated that a single treatment of DEBIRI was comparable to systemic neoadjuvant chemotherapy in terms of tumor response and overall survival[48]. Interestingly, one study on DEBIRI examined outcomes stratified by left-sided or right-sided primary colorectal cancer and found that left-sided colorectal cancer was associated with median OS of 33 mo, while median survival in right-sided colorectal cancer was only 17 mo[49].

TARE in CRLM

Transarterial radioembolic therapy with yttrium-90 has shown a survival benefit in unresectable CRLM. The MORE trial, a retrospective study of 606 patients with unresectable CRLM refractory to one or more lines of chemotherapy, demonstrated that treatment with TARE resulted in median OS of 10 mo[50]. SIRFLOX, a phase III trial, was designed to compare standard systemic chemotherapy (FOLFOX +/- bevacizumab) with systemic chemotherapy plus TARE. Results from this trial showed that the addition of TARE was associated with comparable survival, longer progression free survival, and better tumor response rates in the liver[51]. Further, adding TARE to systemic chemotherapy is associated with less viable tumor tissue after treatment and greater gains in resectability of primarily unrespectable tumors than systemic chemotherapy alone[13,52]. Differences in outcomes between right and left-sided colorectal cancers have been demonstrated in TARE. Of note, TARE added to first-line FOLFOX was associated with a 4.9 mo increase in median OS compared to chemotherapy alone, a difference that was not seen in patients with left-sided primary tumors[53]. Further phase III trials are currently underway to assess the benefit of adjunctive TARE with second-line chemotherapy in CRLM[54].

Ablation in CRLM

Ablation has been long studied in CRLM and demonstrates comparable outcomes to resection when used for small tumors (< 3 cm) with appropriate margins (> 5 mm) [37]. Phase II trials on unresectable colorectal liver metastases revealed that the addition of RFA to systemic chemotherapy increased OS at eight years to 35.9% from 8.9% with chemotherapy alone, with a median OS of 45.6 mo[55]. Recent work has demonstrated the non-inferiority of RFA and MWA for treatment of small resectable liver metastasis[56-58]. These results led to the ongoing COLLISON trial, a rando-

mized, controlled phase III trial comparing overall survival in RFA *vs* surgery in resectable CRLM < 3 cm. Initial discussion suggests there may be a particular role for radiofrequency ablation in small but deep-seated tumors, which would require major hepatectomy using traditional surgical approaches[45]. If shown to be non-inferior, there would be many benefits to adopting a minimally invasive approach like ablation, including decreased morbidity and mortality, length of hospital stay and recovery time. Regarding MWA, recent metanalyses suggest superiority over RFA for resectable CRLM: MWA was found to have similar adverse effect profile and may be associated with increased overall and disease-free survival[59].

Future directions

The five-year survival rate for CRLM has been increasing in recent decades, in part due to locoregional approaches that have enabled downstaging and curative resection in previously unresectable patients. With the recent therapeutic options like DEBIRI and the further development of ablative therapies such as MWA, which can be combined with various chemotherapy regimens, these outcomes will hopefully continue to improve. Other therapies currently under investigation include new ablative techniques like irreversible electroporation[45], for which a phase II trial is currently underway (NCT02082782)[60]. While the role for interventional liver-directed techniques continue to expand, additional research is needed regarding the application of these therapies in an adjuvant setting to improve the multidisciplinary care of CRLM and reduce recurrence rates.

NEUROENDOCRINE TUMORS

Introduction to neuroendocrine tumors with liver metastasis

Neuroendocrine tumors (NETs) are a diverse group of neoplasms that arise from neuroendocrine cells in various parts of the body. They are primarily classified by histology and are generally separated into two groups: indolent, well-differentiated tumors, and more aggressive, poorly differentiated carcinomas. NETs can also secrete hormone peptides, resulting in systemic syndromes. The liver is the most common site of metastasis and the majority of patients with metastatic NETs have neuroendocrine liver metastasis (NELM). Treatment options include surgery, locoregional therapies, chemotherapy, somatostatin analogs, and liver transplant in select patients. After failure of somatostatin analogs, resection is the preferred method of treatment in liver-predominant disease, but curative resection is only possible in 10%-25% of patients and recurrence occurs in 50%-95% of patients[61,62]. Ablation can be used as curative therapy and for downstaging of previously unresectable disease. In patients who are not surgical candidates, typically due to bilobar multifocal disease, locoregional therapies including TAE, TACE, and TARE are the preferred approach for tumor control and management of carcinoid symptoms[62]. Even in the absence of complete disease eradication, locoregional therapies can achieve complete remission of symptoms due to hormone peptide secretion.

TAE and TACE in NELM

TAE and TACE are the preferred therapies for well-differentiated, unresectable, liver-dominant NELM with symptoms that are refractory to medical therapy[19]. In a direct comparison, TAE and TACE were associated with similar outcomes, but TAE was associated with fewer adverse effects than TACE[63]. Dermine *et al*[61] pooled the results of 25 retrospective studies (1986-2017) that examined TACE in NELM and found a progression-free survival (PFS) of 18.5 mo with median OS of 34.5 mo. Measures of response rate were variable, but overall the morphological response rate was 49%, with an additional 27% showing tumor stabilization[61]. In a more recent retrospective study, 197 patients with NELM treated with TACE demonstrated a 96% response by RECIST criteria with a median OS of 35.9 mo and PFS of 15.9 mo[14]. In a comparison between c-TACE with cisplatin, mitomycin C and doxorubicin and DEB-TACE with doxorubicin, c-TACE was associated with higher symptomatic response (47% *vs* 30%) but a higher rate of post-embolization and LFT elevations[64]. Currently, there is an ongoing prospective randomized trial comparing TAE, c-TACE, and DEB-TACE which recently closed its DEB-TACE arm based on initial safety data[65].

TARE in NELM

One analysis which pooled 15 retrospective studies of NELM treated with TARE from

2008 to 2016 found a median symptom response of 89.5% (range: 55-100%), median response rate of 51% (range: 12%-73%) by RECIST criteria, PFS of 10 mo (range: 9-11 mo), and median OS of 28.5 mo (range: 14-70 mo)[61]. The largest study to date included 148 patients with NELM who were treated with TARE and demonstrated a response rate of 70% with a median OS of 70 mo[66]. More recent studies include a retrospective study of 30 patients with NELM who were treated with TARE for a median OS for 39 mo[67]. In one retrospective study, 51 patients with NELM were treated with TARE and demonstrated 83% response by RECIST with median OS of 50.1 mo and PFS of 19.9 mo[14]. A randomized controlled pilot study of 11 patients compared TAE to TARE and found similar response rates by RECIST criteria at 6 mo [68]. A recent multi-institutional analysis found that both TACE and TARE were safe and effective liver-directed therapies for unresectable NELM. Although TACE demonstrated improved short-term disease control and response rates, both resulted in comparable long term outcomes[14].

Ablation in NELM

Ablation can be used alone or in conjunction with surgical resection. When used in conjunction with resection, it can both widen the candidates for resection and provide debulking in bilobar disease. Retrospective study of 16 patients who had a median of 23 liver metastases each were treated with resection and RFA and achieved a 3-year OS of 86 percent[69]. Another retrospective study of 40 patients treated with resection and RFA achieved PFS of 22 mo and median OS of 95 mo[70]. These findings are supported by a third retrospective study of 94 patients who underwent resection with intraoperative ablation, achieving a 5-year OS of 80% and 10-year OS of 59%[71]. Indeed, a recent population-based study found that 30% of patients undergoing resection of NELM also had concomitant ablation with no increase in perioperative morbidity[72]. In some cases, RFA may be as effective as surgical resection; a prospective study of 89 patients with NELM who were treated with RFA alone demonstrated symptom relief in 97% of patients, with a PFS of 16 mo and median OS of 72 mo. Further, the 5-year survival rate of 57% in this study is comparable to the 5-year survival rate of 61% seen in surgical resection[73]. MWA with or without concomitant resection has also been studied for NELM. In a phase II trial of 11 patients, complete ablation, defined as lack of enhancement on triple phase CT, was achieved in 90% of patients at 5 years[74].

Future directions

In addition to locoregional approaches, advances in the molecular understanding of neuroendocrine tumors has led to growing interest in the use of small molecule inhibitors for the treatment of neuroendocrine tumors. Sunitinib, a tyrosine kinase inhibitor, and everolimus, an mTOR inhibitor, have already been approved for use in neuroendocrine tumors[62,75]. An exciting new therapy for neuroendocrine tumors is peptide receptor radionucleotide therapy (PRRT), in which radionucleotides bound to SSA are delivered directly to somatostatin receptor positive tumors[76,77]. In fact, a phase 2 study of TARE with holmium-166 following PRRT is currently underway[78]. Additional research combining locoregional approaches with new therapeutics is needed to explore the benefits in the setting of liver-predominant disease.

BREAST CANCER

Introduction to breast cancer with liver metastasis

Breast cancer is a leading cause of mortality worldwide. Roughly 1 in 5 women with breast cancer will develop metastatic disease to the liver[12,79]. Breast cancer with liver metastasis (BCLM) typically occurs late in the disease course and is associated with a worse prognosis than metastasis to other sites like brain or bone. With treatment, median OS in BCLM is 14 mo[80]. For metastatic disease, systemic therapy remains the standard of care. For patients with isolated BCLM who respond to systemic chemotherapy, surgical resection can be offered[81]. However, recurrence rates even in highly selected patients remain high and the vast majority of patients harbor unresectable disease[12]. Given these limitations, local options like TAE, TACE, and TARE have been used for palliation and to enhance locoregional control.

TACE for BCLM

TACE is a palliative option for BCLM and has shown benefit as an adjunct to systemic

chemotherapy in retrospective studies. Li *et al*[82,83] compared DEB-TACE plus systemic chemotherapy with systemic chemotherapy alone in 47 patients, which demonstrated a median OS of 28 mo, the highest to date for TACE. This is consistent with more recent work by Duan *et al*[84] in 44 patients with liver-only metastatic disease, which demonstrated improved response rates (59.1% *vs* 34.9% by RECIST criteria) and improved survival at 1, 2, and 3 years. In the largest study to date, Vogl *et al*[85] demonstrated a median OS of 25-mo in 208 patients treated with c-TACE and systemic chemotherapy. More recently, a pilot study of DEB-TACE demonstrated disease control and median OS of 17 mo in 23 patients with chemo-resistant disease, though the treatment protocol was associated with adverse effects[86].

TARE for BCLM

TARE is an alternative palliative treatment with promising response rates in chemo resistant BCLM. A study of 81 patients with unresectable liver metastases demonstrated a median OS of 8 mo and a 61% response rate by PERCIST criteria[87]. Most recently, Deipolyi *et al*[88] demonstrated a response rate of 75% at 3-5 mo (PERCIST) and median OS of 15 mo. A recent review of 47 patients who received either TARE or TACE found that TARE was significantly better tolerated and demonstrated a trend toward improved survival. In this study, TARE was associated with a median OS of 13 mo and 3-month disease control in 47% of patients by mRECIST criteria[79].

Ablation for BCLM

For small isolated metastases, ablative therapy may be associated with similar survival outcomes with fewer adverse events compared to surgical resection[12,83]. Recent studies demonstrate median OS ranging from 30 to 70 mo[83]. One retrospective study of 69 patients with BCLM demonstrated PFS of 24 mo, with median OS of one-, two-, three- and five-year survival rates of 81.8, 50.1, 25.3 and 11.0%, respectively[89]. A more recent retrospective study of 33 patients with oligometastatic breast cancer demonstrated a median OS of 70 mo. Subgroup analysis of 14 patients with hepatic metastasis revealed PFS of 9 mo, which improved to 13 mo in patients who were able to achieve ablation of all metastatic disease in the liver[90]. Prognostic factors associated with improved tumor control and PFS across multiple trials include tumor size, estrogen receptor positivity, and 5-10 mm ablation margins.

Ablation is an appropriate therapy for tumor control of isolated liver metastases and reduces the need for time on systemic chemotherapy. Prospective randomized trials comparing systemic therapy alone to systemic therapy with ablation are needed to determine whether ablation offers a survival benefit as an adjunctive therapy. Further, given that ablation is associated with similar overall survival and decreased morbidity and mortality compared to resection, prospective randomized trials are needed to compare the two approaches to determine the appropriate standard of care.

Future directions

In addition to recent advances in locoregional strategies, new immunotherapies, pembrolizumab and atezolizumab, have been recently FDA-approved to treat metastatic breast cancer[91,92]. Additionally, the FDA recently approved the use of poly(ADP-ribose) polymerase (PARP) inhibitors olaparib and talazoparib in metastatic breast cancer[93,94]. There is growing interest in the synergistic effects of PARP inhibitors combined with radiotherapy, and additional studies are needed to determine outcomes in BCLM[95]. Future studies comparing combination strategies of immunotherapy, PARP inhibitors, and locoregional therapies like ablation and TARE are needed, as they may be able to demonstrate improved outcomes BCLM and strengthen the multidisciplinary care of BCLM.

LUNG CANCER

Introduction to lung cancer with liver metastasis

Lung cancer is the leading cause of cancer death worldwide and metastatic disease is associated with a 5-year survival rate of 4%. Liver metastasis in particular is associated with a worse prognosis compared to metastasis to the brain or bone and is most common in small cell lung carcinoma[96]. In this setting, treatment consists of palliative systemic chemotherapy. However, the 2018 TMN staging criteria distinguish between single and multiple extra thoracic metastasis, suggesting that a more aggressive approach to limited metastatic disease may improve outcomes. Data on

surgical resection is limited to a handful of case reports, which describe benefit in select patients[97-99].

Locoregional therapies for lung cancer with liver metastasis

The benefit of local therapies like TACE, TARE, and ablation are not well characterized for lung cancer with liver metastasis. Regarding TACE, review of a prospective multi-institutional registry containing 13 patients with liver metastasis who were treated with DEB-TACE using either doxorubicin or irinotecan revealed a response rate of 50% at 12 mo and a median OS of 14 mo[100]. Data on TARE is limited to nine patients discussed in two case reports and one retrospective review and demonstrate its potential as salvage therapy in chemo-refractory disease[101]. Regarding ablation, one retrospective review contained four patients with solitary liver metastasis from a primary non-small cell lung cancer who were treated with MWA. Treatment was well tolerated, but subgroup analysis on response rate and overall survival was not performed[102]. Despite the lack of prospective data, local therapies may provide benefit as adjunctive treatment in select patients with oligometastatic disease.

UVEAL MELANOMA

Introduction to uveal melanoma with liver metastasis

Uveal melanoma is the most common primary malignant intraocular tumor in adults. Nearly half of patients will develop metastatic disease, and of those, over 90% will have primary metastasis to the liver. While surgical resection remains the standard of care when feasible, less than 10% of patients will be candidates for surgical resection [10]. Additionally, metastatic uveal melanoma is generally unresponsive to systemic chemotherapy. Without treatment, prognosis for metastatic disease is poor, with median survival of less than nine months[10]. As no medical therapies have yet been shown to prolong survival, there are currently no FDA-approved therapies for metastatic disease. Notably, the major advances in metastatic cutaneous melanoma using immunotherapy have not yet been replicated in metastatic uveal melanoma. Therapeutic approaches under investigation include systemic immunotherapy with checkpoint inhibitors nivolumab and ipilimumab as well as a number of locoregional approaches including ablation, radio- and chemoembolization, and intrahepatic perfusion. The rarity of metastatic uveal melanoma presents a challenge for study design, and many studies comprise cohorts of 20 to 50 patients with wide ranges in outcome measurements between studies.

Locoregional therapies for uveal melanoma with liver metastasis

Recent work by Höppener *et al*[103] provides a meta-analysis of locoregional approaches in uveal melanoma with metastasis to the liver. The vast majority of studies are retrospective cohort studies and outcomes examined include tumor control, progression free survival, and overall survival. In 19 studies of TACE median OS was 6 mo (range 5 to 28). Cisplatin was the most common chemotherapeutic used, and others included doxorubicin, mitomycin-c, fotemustine, and irinotecan. Of these, two studies compared TACE to systemic chemotherapy and found no difference in overall survival[104,105]. Thirteen studies of SIRT with ^{90}Y demonstrated a median OS of 11 mo (range 4-26) and included a recent phase II trial showing median survival of 10 mo [10]. Six studies of ablation (predominantly RFA) demonstrated median OS of 19 mo (range: 11-46) and included a recent phase Ib/II trial that combined RFA with ipilimumab to little clinical effect[106]. Fourteen studies examined intrahepatic perfusion of melphalan, a unique approach which involves the introduction of melphalan *via* the hepatic arteries paired with IVC bypass with melphalan filtration to prevent systemic circulation of chemotherapy. This approach demonstrated a median OS of 11 mo (range 5-27). While comparable in terms of overall survival, unique adverse events have been reported using this approach due to the cardiovascular and coagulopathic risks of bypass.

Future directions

Given the lack of a standard of care for metastatic uveal melanoma, many other approaches are being developed alongside locoregional therapies. In addition to the locoregional approaches above, hepatic artery infusion with fotemustine has shown potential benefit in uveal melanoma with liver metastasis[107]. Seventeen studies of hepatic artery infusion with fotemustine had a median OS of 13 mo (range 3-21). In the

last ten years, the development of immune checkpoint inhibitor therapies including ipilimumab, nivolumab and pembrolizumab have transformed outcomes for malignant melanoma[108]. While these results have not yet been replicated in uveal melanoma, trials of combination therapy have shown some potential benefit[109]. Other experimental agents for uveal metastatic melanoma include tumor infiltrating lymphocytes[110], epigenetic therapies[111-113], and tebentafusp, a bispecific fusion protein that targets CD3+ and T-cell receptors[114]. Additional research is needed regarding the application of these therapies in a neoadjuvant or adjuvant setting to improve the multidisciplinary care of metastatic uveal melanoma.

CHOLANGIOCARCINOMA

Introduction to intrahepatic cholangiocarcinoma

Cholangiocarcinoma is a rare malignancy of the biliary system that is occurring with increasing incidence and can be anatomically divided into intrahepatic, perihilar, and extrahepatic types. Given the aggressive and asymptomatic course of early disease, late presentation is common. While the current standard of therapy is resection, recurrence is seen in 60% of patients[115,116]. Further, about 75% of patients are not candidates for resection at time of presentation due to tumor size, location, multifocality, or distant metastatic disease[117]. In the case of unresectable disease, the current standard of care is systemic platinum-based chemotherapy plus gemcitabine, which confers a median OS 11.7 mo based on the results of the ABC-02 trial[118]. In the decade since this trial, studies have shown benefits associated with locoregional therapies in the treatment of intrahepatic cholangiocarcinoma (IHC), including improved overall survival and successful downstaging to surgical intervention[119].

TACE in IHC

With regard to trans-arterial chemoembolization, both c-TACE and DEB-TACE have been investigated in patients with IHC. Park *et al*[120] demonstrated improved OS in patients treated with c-TACE *vs* supportive care, and meta-analysis of 542 patients with IHC treated with c-TACE reveals a median OS of 13.4 mo after treatment[121]. In a study of 24 patients, DEB-TACE was associated with median OS of 17.5 mo[122], and studies of combination DEB-TACE with systemic chemotherapy have demonstrated higher median overall survival than treatment with chemotherapy alone[123,124]. In a three-way comparison of systemic chemotherapy, c-TACE, and DEB-TACE in patients with IHC, DEB-TACE was associated with greater OS than c-TACE, and similar OS to systemic chemotherapy[125]. The ongoing CTILC study (NCT03317483) investigating DEB-TACE in various liver cancers includes 37 patients with IHC[126].

TARE in IHC

Radioembolization with ^{90}Y plus first-line chemotherapy has been shown to increase overall survival and successfully downstage patients to surgical resection. A recent single-center retrospective study of 85 patients showed median OS of 21 mo from time of diagnosis and median OS of 12 mo after treatment[127]. These findings were further supported by a multicenter retrospective study of 115 patients showing median OS from diagnosis of 29 mo with median OS after treatment of 11 mo[128]. Within the last year, Edeline *et al*[129] published the results of a phase 2 trial of ^{90}Y with first-line chemotherapy in 41 patients with a response rate of 40%, median OS of 22 mo, and successful downstaging of over 20% of trial participants. Given the significant improvement in overall survival compared to the current standard of care, a phase 3 trial is ongoing[129].

Ablation in IHC

Ablation is an option in select patients who are poor surgical candidates and have early-stage IHC (< 5 cm)[130]. Given these criteria, few patients are candidates and available study sizes are small. Recent metanalysis of 10 studies with a total of 206 patients showed median OS for patients treated with RFA ranged from 8.7 to 52.4 mo [131]. Preliminary research suggests that MWA confer a survival benefit *vs* RFA in tumors less than 3cm[132]. Given the invasive nature of the disease, multiple authors recommend wide ablation margins[133-135]. No studies have specifically investigated the role of cryoablation or irreversible electroporation for IHC[136].

Future directions in IHC

Given the importance of chemotherapy in the treatment of IHC, ongoing trials of DEB-TACE represent an exciting area of research. Additionally, the development of targeted therapies for IHC is an area of active research and may eventually be used in conjunction with locoregional approaches to improve outcomes. Currently, phase III trials of the isocitrate dehydrogenase 1 (IDH-1) inhibitor ivosidenib and fibroblast growth factor receptor (FGFR) inhibitors are ongoing[117]. Finally, there are promising results from studies of hepatic artery infusion with floxuridine (FUDR-HAI) combined with first-line chemotherapy[137-140]. While the role of interventional liver-directed therapies continues to expand, it remains to be seen how new targeted approaches can be combined with locoregional strategies to improve multidisciplinary care of IHC.

SARCOMA

Introduction to sarcoma with liver metastasis

Sarcomas are a diverse set of tumors that arise from mesenchymal cells in various parts of the body. These mesenchymal cells can differentiate into a variety of tissues including muscle, adipose, cartilage, nerve, and vascular tissue. Prognosis is related to tumor type — gastrointestinal stromal tumors, for example, are associated with better prognosis, while leiomyosarcomas, which are notoriously resistant to systemic chemotherapies are associated with poor prognosis[141]. In all types, the feared complication is hematogenous metastasis, which is considered incurable and associated with median survival of 12 to 19 mo. In metastatic disease, palliative chemotherapy is the standard of care, despite the fact that only 10-25% of metastatic sarcomas respond to systemic chemotherapy[142]. There is growing interest in more aggressive local treatment, especially for oligometastatic disease. While complete surgical resection is preferred, many patients are not surgical candidates. In unresectable, recurrent or chemo-resistant disease, local therapies like TAE, TACE, and ablation are associated with increased tumor response and overall survival.

TAE in sarcoma with liver metastasis

Two studies of TAE demonstrated improved response rate and overall survival in patients with unresectable, chemoresistant sarcoma with liver metastasis (SLM). The first was a retrospective study of patients with hepatic metastasis that was either incompletely resectable or had failed other therapies. Treatment response was defined as greater than 25% reduction in tumor size or greater than 50% necrosis and achieved response in 9 of 15 patients. OS was 62%, 41%, and 29% at 1-, 2-, and 3 years respectively[143]. The second study examined TAE in 11 patients with GIST that had metastasized to the liver in patients who had either been treated with first line imatinib alone or first-line imatinib followed by and second line sunitinib. In the first group, median survival was 15 mo and PFS was 3.8 mo. In the second group, TAE achieved a median OS of 24 mo and PFS of 3.4 mo. Response rate was 46% overall by mRECIST criteria[144]. GIST tumor type and radiographic response were both associated with prolonged survival. These results represent improvement in both response rate and overall survival compared to treatment with second- or third-line chemotherapy.

TACE in SLM

Three retrospective studies examine the use of TACE in sarcomas with liver metastasis. The earliest, in 1995, used cisplatin beads with vinblastine arterial infusion in 14 patients with gastrointestinal leiomyosarcoma with prior resection. However, local therapy with cisplatin and vinblastine induced > 50% reduction in tumor size in 70% of patients with median PFS of 12 mo[145]. These findings are supported by a retrospective review of 16 patients, most with leiomyosarcoma, which demonstrated tumor control or response in 83% of patients and a median OS of 20 mo after treatment with cisplatin, doxorubicin, and mitomycin-C[146]. Most recently, a retrospective study of 30 patients treated with c-TACE using doxorubicin, cisplatin, and mitomycin-C demonstrated a response of 48% by mRECIST criteria, PFS of 6.3 mo, and median OS of 21 mo[142]. These studies reveal that TACE is an appealing option, particularly in the treatment of leiomyosarcomas, which are highly resistant to systemic chemotherapy.

Ablation in SLM

Ablation for SLM has been examined in three retrospective studies. The first included 66 patients with SLM who were treated with either surgical resection, RFA, or combination therapy. Of the 18 patients who underwent surgical resection with RFA and 13 patients who underwent RFA alone, PFS was 7.4 mo and median OS was 33.2 mo [147]. The second retrospective study comprised 13 patients with GIST with liver metastasis and 12 patients with other sarcoma subtypes with liver metastasis. Of the patients with GIST, 85% showed tumor response with a single treatment of RFA, and non-responders were treated with a second round of RFA, achieving total response. Patients with GIST demonstrated PFS of 28 mo. In other tumor types with liver metastasis, response was observed in 71% of patients, with PFS of 7 mo[148]. Most recently, data from a large retrospective study of 281 patients with metastatic sarcoma support the use of RFA in non-resectable metastatic disease[149]. In addition to these retrospective studies, there are a number of recent case reports on RFA in SLM[150-152]. Ablation is generally well-tolerated and is associated with greatly improved tumor response, progression free, and overall survival in patients, particularly in patients with unresectable or chemo resistant SLM. Further, RFA and surgery can be used in conjunction in many sarcoma subtypes to maximize outcomes.

Future directions

Given the diversity of sarcomas, there is ongoing research into a number of small molecule inhibitors for specific sarcoma subtypes[153]. For metastatic sarcoma in general, preliminary research demonstrates promising outcomes with tivozanib, a VEGF inhibitor[154], and a new chemotherapeutic, eribulin, which has demonstrated benefit in combination with dacarbazine[155]. Additional studies exploring the use of these therapies in conjunction with DEB-TACE would elucidate the role for these therapies in liver-predominant disease.

CONCLUSION

Metastatic disease to the liver is the most common malignant liver condition and a major cause of cancer-related morbidity and mortality. Surgical resection and systemic chemotherapy remain the standard of care in most types of metastatic liver disease, but there is an expanding role for locoregional therapies in liver metastasis with various aims including curative intent, tumor control, downstaging to resection, symptom control, and palliation. TAE, which can be combined with chemotherapy and/or radiotherapy, has the potential to improve tumor response rates and disease-free and overall survival in select patients. Ablative procedures using high frequency alternating currents or microwaves represent comparable alternatives to resection and can even achieve curative results in selected patients. Combined with advances in immunotherapy and targeted therapies, advances in locoregional approaches are providing more robust, multidisciplinary treatment options for metastatic liver disease.

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REVIEW

Hematopoietic stem cell mobilization strategies to support high-dose chemotherapy: A focus on relapsed/refractory germ cell tumors

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Abstract

High-dose chemotherapy (HDCT) with autologous hematopoietic stem cell transplantation has been explored and has played an important role in the management of patients with high-risk germ cell tumors (GCTs) who failed to be cured by conventional chemotherapy. Hematopoietic stem cells (HSCs) collected from the peripheral blood, after appropriate pharmacologic mobilization, have largely replaced bone marrow as the principal source of HSCs in transplants. As it is currently common practice to perform tandem or multiple sequential cycles of HDCT, it is anticipated that collection of large numbers of HSCs from the peripheral blood is a prerequisite for the success of the procedure. Moreover, the CD34+ cell dose/kg of body weight infused after HDCT has proven to be a major determinant of hematopoietic engraftment, with patients who receive > 2×10^6 CD34+ cells/kg having consistent, rapid, and sustained hematopoietic recovery. However, many patients with relapsed/refractory GCTs have been exposed to multiple cycles of myelosuppressive chemotherapy, which compromises the efficacy of HSC mobilization with granulocyte colony-stimulating factor with or without chemotherapy. Therefore, alternative strategies that use novel agents in combination with traditional mobilizing regimens are required. Herein, after an overview of the mechanisms of HSCs mobilization, we review the existing literature regarding studies reporting various HSC mobilization approaches in patients with relapsed/refractory GCTs, and finally report newer experimental mobilization strategies employing novel agents that have been applied in other hematologic or solid malignancies.

Key Words: Hematopoietic stem cells; Germ cell tumors; Hematopoietic stem cell transplantation; Granulocyte colony-stimulating factor; Plerixafor

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Core tip: High-dose chemotherapy (HDCT) followed by autologous stem cell transplantation (ASCT) is a curative treatment option for patients with relapsed/refractory germ cell tumors (GCTs). Mobilization of adequate numbers of hematopoietic stem cells (HSCs) is a prerequisite for successful ASCT. As the benefit of HDCT+ASCT is largely evident with > one HDCT cycle, it is anticipated that an appreciable percentage of patients will not mobilize adequate HSCs and require salvage strategies. Herein, we review the history of HSC transplantation, with emphasis in GCTs, pathophysiological mechanisms of HSC mobilization, initial and salvage mobilization strategies, and finally discuss novel mobilizing agents and approaches to overcome failures.

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INTRODUCTION

High-dose chemotherapy (HDCT) followed by autologous hematopoietic stem cell transplantation (ASCT) has been a major breakthrough in oncology. It has broad applicability in patients with metastatic germ cell tumors (GCTs) who experience one or even more relapses after previous chemotherapy, or in those with a poor prognosis on diagnosis (*e.g.*, with extragonadal primary or incomplete response to first-line cisplatin-based chemotherapy)[1,2]. The efficacy of HDCT and ASCT depends largely on successful and adequate hematopoietic stem cell (HSC) mobilization, which ensures faster neutrophil and platelet engraftment and therefore decreased infection risk and hospitalization[2]. Collection of at least 2.0 × 10^6 CD34+ HSCs has been considered the minimum for a subsequent successful ASCT[3,4]. However, successful mobilization remains a great challenge, as a significant number of patients, somewhere between 5%-30%, are unable to mobilize enough HSCs to support subsequent ASCT. That has been attributed to extensive and prolonged prior exposure to bone marrow-suppressing intensive chemotherapy that has ultimately led to poor bone marrow reserves [5]. Indications, as far as strategies appropriate for achieving adequate CD34+ cell numbers for these patients, are limited by a lack of data and are generally based on standard approaches for HSC mobilization that have been applied in other disease settings. Hence, the establishment of standard mobilization and remobilization techniques for patients with GCTs who failed the initial mobilization protocols should become a high priority (outlined in Figure 1).

GERM CELL TUMORS

Testicular cancer and GCTs typically subdivided into two main histologic subtypes, seminomas and non-seminomas, are the most common solid tumor in men between 20 and 35 years of age[6,7]. Approximately 50% of testicular cancers are non-seminomas, which are typically more malignant and usually associated with a more aggressive clinical presentation[8]. The cure rates are between 41%-92%[9,10]. About 20%-30% of patients with metastatic disease at initial presentation will eventually require salvage treatment. Second-line therapy options include conventional dose cisplatin-based regimens, or high-dose chemotherapy regimens, currently consisting of carboplatin and etoposide plus ASCT support[10,11].

To date, the main conventional dose chemotherapy (CDCT) salvage regimens include etoposide-ifosfamide-cisplatin, vinblastine-ifosfamide-cisplatin, and paclitaxel (taxol)-ifosfamide-cisplatin (TIP)[12,13]. Randomized data are lacking, and retrospective comparisons have failed to demonstrate the superiority of any of these regimens. Nevertheless, the best results were observed with TIP, which is therefore currently broadly accepted as the optimal choice of salvage chemotherapy.

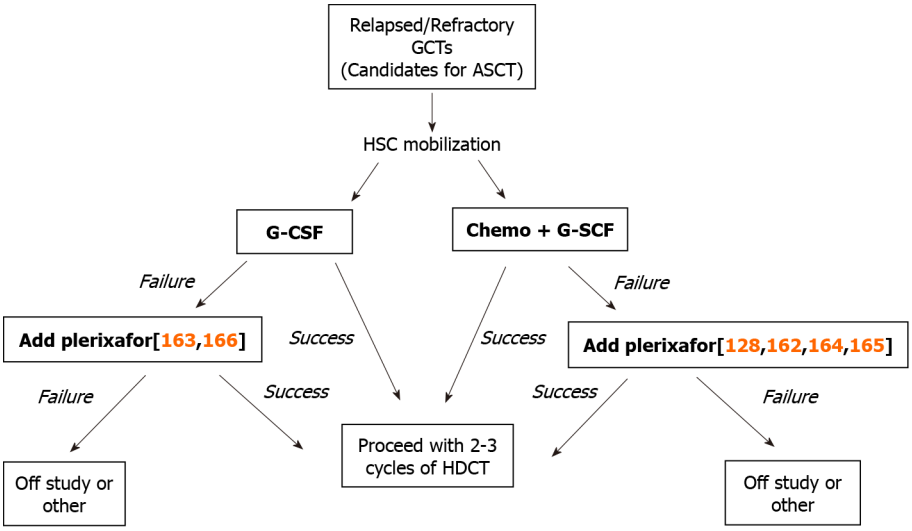

Figure 1 Mobilization algorithms. ASCT: Autologous stem cell transplantation; G-CSF: Granulocyte colony-stimulating factor; GCTs: Germ cell tumors; HDCT: High-dose chemotherapy; HSC: Hematopoietic stem cell.

CURRENT STATUS OF HDCT AND ASCT IN GERM CELL TUMORS

In HDCT, cytotoxic agents are administered at much higher doses than the standard dose applied in CDCT. The observation of a larger therapeutic impact even at minor increases of dosage, proved the dose-response relationship of many chemotherapeutic agents, and thus supported the efficiency of HDCT regimens in eradicating residual drug-resistant tumor cells[14]. Increased doses lead also to more severe side effects, with prolonged myelosuppression being the main reason to delay subsequent cycles, thus leading to failure[15]. To reduce the duration of pancytopenia, and therefore the failure rate, HSCs are harvested from the patient's peripheral blood by apheresis before the administration of HDCT. After completion of HDCT the harvested stem cells are reinfused to repopulate the bone marrow and ultimately re-establish hematopoiesis. Despite the fact that the use of HDCT as salvage in GCTs is a standard treatment option for most patients, its efficacy as a first salvage strategy remains a matter of debate among investigators[16-19]. An ongoing phase III trial - the TIGER study - may be the first to establish HDCT as initial salvage in these patients, considering the existing inconsistent evidence as well as the lack of conclusive randomized trials.

HISTORY OF ASCT

Total-body irradiation (TBI) prior to autologous transplantation was first applied in animals in the 1930's. The early studies had fatal outcomes because of severe gastrointestinal and nervous system complications, hemorrhage, and infection[1,2]. Similar trials of TBI were performed in humans few years later. The first was performed by Thomas and his colleagues in a leukemic patient, who was grafted with bone marrow from her identical twin sister. They reported a 3-month remission duration in this patient. Following the discovery of the human leucocyte antigen (HLA) system by Dausset in 1958[20], the concept of histocompatibility, *i.e.* identical HLA in both the donor and recipient (patient),was applied, with high success rates for allogeneic transplantations.

STEM CELL SOURCES-DIFFERENCES BETWEEN PERIPHERAL BLOOD HSCs AND BONE MARROW HARVESTING

Bone marrow was the first source of HSCs, which were obtained by repeated aspirations from the posterior iliac crests with the donor under general or local anesthesia. The method was used for many years until the observation that stem cells detach, enter the circulation and home to the marrow. After that observation, peripheral blood

harvesting, as more convenient and appropriate source of HSC, has replaced bone marrow[1]. There are two types of peripheral blood leukapheresis, normal volume and large volume. The normal volume procedure processes 2.5 to 3 times the patient blood volume. The large volume procedure processes 4-5 times the volume. Many researchers evaluated the efficacy and safety of large volume leukapheresis and concluded that, after successful mobilization, this leads to a higher CD34+ cell harvest without a change in graft quality ,with fewer sessions to reach greater than 2 × 10^6 CD34+ cells/kg body weight[3,4,21].

Goldman *et al*[22] was the first to use HSCs collected from the peripheral blood for autologous transplantation after high-dose cytotoxic therapy in patients with CML. Körbling *et al*[23] followed with a report of autologous transplantation in a patient with CML, and a patient with Burkitt's lymphoma. Körbling *et al*[23] reported the collection of peripheral blood stem cells after the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) during leukocyte recovery after myelosuppressive chemotherapy. That was the first example of chemotherapy-induced "mobilization". Subsequently Kessinger *et al*[24] used the same mobilization method and documented that performing multiple leukapheresis sessions resulted in a sufficient number of circulating HSCs in the peripheral blood to ensure engraftment after HDCT.

DIFFERENCES BETWEEN PERIPHERAL BLOOD HSC AND BONE MARROW HARVESTING

Traditionally, as HSCs reside in the bone marrow at steady-state conditions, collection has been carried out by bone marrow harvesting from the posterior iliac crests and possibly the sternum under general or epidural anesthesia[25]. Bone marrow harvesting, as mentioned earlier, is a one-time procedure with multiple risks that increase with donors age and comorbidities. Peripheral blood HSC (PBSC) collection performed by large-volume leukapheresis, is dependent on stem cell mobilization, and a prolonged harvesting period is required. However it is considered safe to perform on donors without the need of any type of anesthesia. A limitation of PBSC collection is adequate venous access. PBSC collection performed by single or multiple apheresis avoids the risks of general anesthesia and shortens the time for hematopoietic recovery. The most common adverse effects include moderate-to-severe bone pain as a result of leucocyte growth factor administration, fatigue, and headache. Rare adverse events include splenic rupture, acute arthritis, anaphylaxis, and cardiac ischemia[26-28].

Since the early 90's, HSCs mobilized from the bone marrow into the peripheral blood (PB) have been established as the preferred source of HSCs for transplantation because they are easily accessible, and the evidence indicates that they engraft faster after transplantation than HSCs directly harvested from bone marrow (BM). Clinical findings from randomized/comparative trials indicate that patients experience faster neutrophil, platelet, and immune recovery after PB stem cell transplantation; and in allogeneic transplantation, a higher incidence of chronic graft *vs* host disease and lower probability of relapse[29].

HSCS MOBILIZING AGENTS

HSCs are multipotent precursors with self-renewal potency that reside predominantly in the bone marrow. A small number of HSCs circulate in the blood (< 0.02%) under steady-state conditions[30]. Several methods have demonstrated effectiveness in increasing the percentage of HSCs in PB and maximize the number collected with the intention of restoring marrow function and reduce the time required for neutrophil and platelet engraftment following HDCT. Initial mobilization strategies include: (1) Administration of hematopoietic CSFs alone; (2) A course of myelosuppressive chemotherapy prior to collection; and (3) Chemotherapy followed by cytokine administration. Remobilization strategies include: (1) Dose escalation of leucocyte CSFs; granulocyte (G)-CSF or granulocyte-macrophage (GM)-CSF, with or without IL-3; (2) Different forms of G-CSF, with altered glycosylation patterns to improve pharmacokinetics and bioavailability; (3) G-CSF in combination with other HSC mobilizing agents, *i.e.* Plerixafor or stem cell factor (SCF), *kit*-ligand (known as ancestim); and (4) G-CSF in combination with chemotherapy and newer agents like plerixafor. A course of myelosuppressive chemotherapy prior to HDCT as a chemo-mobilization strategy

not only increases stem cell collection, but also provides better control of the underlying malignancy, when active agents or chemotherapy regimens are administered [31,32]. However, an increased risk of infection and hospitalization is expected in patients undergoing chemo-mobilization[31].

In turn, the administration of mobilization agents alone not only has the benefit of relatively predictable kinetics of mobilization, but also a reduced need for hospital care compared with chemotherapy because of the minimal side effects of G-CSF[33,34]. The most commonly used myeloid growth factor for peripheral stem cell harvesting is G-CSF. Other alternatives are its pegylated form; pegfilgrastim, and sargramostim; the recombinant human GM-CSF. Several studies now confirm higher successful rates and twice as many progenitor cells in the circulation when a combination of chemotherapy and G-CSF is used. Consequently, that approach is favored by many investigators[35, 36].

Having said that, the use of newer agents, such as chemokine receptor antagonists, along with the conventional ways of autografting mentioned above has expanded in recent years, with promising synergistic results. Plerixafor, a bicyclam molecule derivative that reversibly competes with and inhibits stromal-derived factor-1a (SDF-1a; also known as CXCL12) binding to CXCR4, causes an absolute peak of CD34+ cells 6-9 h after administration. Administration is preferable in the evening before apheresis, ideally 8-10 h before the procedure to maximize the number of HSCs collected [37]. Daily administration of plerixafor in the evening for up to four consecutive days can be given, with a morning G-CSF dose along with the apheresis sessions if the desired HSC target number has not been achieved[38]. However, considering the higher cost of that approach, one recognizes the need to establish specific mobilization algorithms in order to maximize the potential of the conventional mobilization agents. That improves the pharmaco-economics of mobilization and reduces the need of rescue remobilization with plerixafor. Nowadays, because of its high cost, plerixafor use is restricted to patients failing to reach sufficient PB CD34+ cell counts (*i.e.* preemptive application) on the day that apheresis is planned to start or in patients failing to collect sufficient CD34+ cells during leukapheresis (*i.e.* rescue application). Preemptive use of plerixafor, especially in combination with G-CSF in poor mobilizers has proven to be more cost effective[39,40].

MOBILIZATION ALGORITHMS TO OPTIMIZE MOBILIZATION OUTCOMES

In patients with relapsed/refractory GCTs, we and others attempt HSC mobilization preferably after 1 or 2 salvage chemotherapy cycles with TIP or TI followed by the administration of G-CSF between days 3 and 11 or until the day when sufficient numbers of CD34+ HSCs have been obtained. This approach is accompanied by frequent measurement of circulating PB CD34+/μL counts by flow cytometry, usually starting on day 10-11, in order to decide when to perform the apheresis. A mobilization algorithm called the "just in time"[41] approach helps to decide whether the patient is in need of plerixafor. Patients with an absolute number of CD34+ cells > 3 and < 15/μL are the main candidates for plerixafor administration. Other protocols include "one size fits all"[42], in which a standard technique is applicable to all patients and "risk-based approaches"[43]. The latter places patients into categories, where those who meet more of the predefined criteria are more likely to be poor mobilizers, and thus a different approach must be used. Poor mobilizers are defined as those who have received many prior lines and cycles of chemotherapy, particularly those who have been exposed to alkylating agents, irradiation, pre-existing low blood counts, bone marrow involvement by the tumor, and advanced age[39,44].

UNDERSTANDING THE STEM CELL NICHE IS CRITICAL FOR FURTHER PHARMACOLOGICAL STUDIES

Schofield was the first to propose the concept of HSCs in 1978[45]. Since then, many have attempted to virtually define this area[46-49], and as a result, we now refer to stem cell niche as the microenvironment where localization and regulation of stem cells takes place. The area is anatomically located near to the endosteum and is composed by two major compartments, the perivascular and the endosteal niches, where cells and molecules dynamically interact[50,51]. The endosteal niche compartment consists of osteoblasts and is critical for supporting the lymphoid progenitors

[52]. It is a hypoxic environment that favors the undifferentiated state of HSCs[53], where low energy supplies are needed. Hypoxia is a critical component of the HSC niche[54], and exposure of HSCs to elevated oxygen tissues negatively affects self-renewal and promotes cell cycle entry, hindering low-cycling proliferation[54,55]. Low oxygen concentration in the endosteal niche is regulated by hypoxia-inducible factor-1 (HIF-1), a transcription factor, which under hypoxic conditions, binds in its full heterodimeric form (HIF1a + HIF1b) to DNA elements controlling transcription of various genes related to angiogenesis and erythropoiesis, resulting in the upregulation of vascular-endothelial growth (VEGF), which ultimately leads to vasodilation and HSC mobilization[56].

The vascular niche is rich in oxygen, and it is thought that HSCs migrating towards the niche proliferate and regenerate. This compartment is subcategorized into arterial-perivascular, mesenchymal, and sinusoidal endothelial niches. Recent studies showed that the arterial-perivascular niche mostly consists of nestin-bright (nestin+)-smooth muscle perivascular cells[57,58] that express high levels of CXCL12/SDF1 under steady-state conditions and therefore appear to be strongly associated with both proliferation and maintenance of primitive hematopoietic cells in a quiescent state[58,59]. The endothelial sinusoidal niche is composed of endothelial cells that are nestin-dim/leptin receptor-2 (LEPR2) and CXCL12-abudant reticular (CAR) cells with high amounts of CXC-L12, which contribute to regeneration after myelotoxic stress[58]. Several studies showed that as HSCs enter the cell cycle they relocate from areas rich in nestin-bright perivascular cells to those rich in LEPR2+ cells and are mobilized into the circulation[58-60]. In addition to cellular interactions, stem cells are attracted to the bone marrow niche cells through dynamic interactions involving soluble factors (*e.g.*, growth factors, chemokines and cytokines, and adhesion molecules).

One of the most critical chemotactic factors, SDF1a (CXCL12), mainly derived from osteoblasts and endothelial cells, attract HSCs by attaching to their surface chemokine receptor; CXCR4[61]. Other important adhesion molecules are VCAM1 (CD106), which binds to integrin α4β1, very late antigen-4 (VLA-4) on HSCs, and a transmembrane SCF that binds to c-kit (CD117) on HSCs[62,63]. It is well understood that the breaking down of those tethers is necessary for the release of HSCs into the circulation.

Other cells, such as adipocytes, and macrophages have supporting roles in the BM environment. CD169 macrophages secrete oncostatin-M, which leads to increased CXCL12 production by nestin+ and other mesenchymal cells *via* the MAPK-p38 signaling pathway[64,65]. Depletion of the macrophages results in downregulation of VCAM1, SDF1a, and SCF expression that disrupts the normal niche functions[64,65]. The percentage of adipocytes in the BM, derived from mesenchymal cells, increases with age, leading to a fatty marrow with limited cell proliferation ability[66].

INITIAL MOBILIZATION STRATEGIES

*Use of G-CSF or biosimilar**

Brief history: In 1966, Ray Bradley and Don Metcalf were the first to identify agents that can stimulate colony formation in hematopoietic cells in semi-solid culture[67]. Later, in 1985 Welte *et al*[68] purified human G-CSF. Nagata *et al*[69] in Japan and independently Souza *et al*[70] from AMGEN in 1986 cloned the G-CSF gene, resulting in the production and clinical application of this cytokine. The first preclinical data to demonstrate mobilization of hematopoietic cells following the administration of G-CSF in mice was in 1986 in a study conducted by Tamura *et al*[71], where an observation of increasing neutrophil counts approximately 2 h after injection made. The following year, Duhrsen *et al*[72], confirmed the mobilizing activity of G-CSF in cancer patients, where an increase of mature and progenitor cells into the circulation was observed. The observations were the stimuli for further animal studies to determine whether the progenitor cells could be effective for hematopoietic reconstitution[73].

Mechanism of action: The G-CSF receptor (G-CSFR) is expressed on a range of hematopoietic cells, including mature neutrophilic granulocytes, myeloid progenitors, and HSCs[74]. After binding to its ligand, receptor multimerization and activation of several intracellular signaling cascades occur, including the Jak/Stat/Socs, Ras/Raf/Erk and PI3-kinase/Akt pathways, which ultimately leads to transcriptional changes that have an impact on survival, migration, proliferation, and differentiation[74]. G-CSFR signaling also mediates the mobilization of hematopoietic progenitor cells (HPCs) and mature neutrophilic granulocytes from the bone marrow[75]. Multiple mechanisms have been described to explain the mechanism of action of G-CSF.

Because most of the topics are still poorly understood, further studies are required. It has been previously hypothesized that the mechanism of mobilization by G-CSF is indirect, based on the fact that HSCs themselves, in order to mobilize, do not express the G-CSFR receptor[76], which is mainly expressed on the surface of macrophages and osteomacs[77]. (1) The first mechanism includes the role of proteases. It is known that following G-CSF administration, an increase in the number of granulocytes occurs. The increase is accompanied by the production of large amounts of proteases such as neutrophil elastase, cathepsin, and MMP-9 by neutrophils[78], which in combination with other proteases, such as the CD26 dipeptidase[79], inactivate multiple adhesion molecules (VCAM1, CXCR4, fibronectin, c-kit, SCF, OPN), thereby disrupting their attachment to the VLA4 receptor and weakening intracellular adhesive interactions[80-83]. One of the most important mechanism is the induced proteolytic clearance and degradation of SDF1 (CXCL12) in the bone marrow. Matrix metalloproteinase (MMP)-9[84,85] and CD26 cause the cleavage of the NH2-terminal of SDF1, so it can no longer contact the surface CXCR4 receptor, leading to liberation of HSCs into the circulation[80,86]. In addition, type 1 metalloproteinase (MMP1) increases CD44 cleavage. CD44 ligand is hyaluronic acid, rich in endosteum and sinusoidal endothelium, and essential for HSCs homing[87]. (2) The second involves changes in bone formation. Following G-CSF administration, a variety of changes in bone formation occur, more specifically an almost complete loss of the osteoblastic layer has been observed[65,75,88]. Osteoblasts are essential in the BM microenvironment by producing cytokines, chemokines and adhesion molecules[89]. The osteoblasts, however, do not express the G-CSFR[88,90], which suggests that this effect is mediated by other cell types. Osteoclasts arise from HSCs and do express the G-CSF receptor, so it has been proposed that they play a critical role not only in formation of the hematopoietic niche, but also in HSC mobilization through secretion of cathepsin K, which cleaves and inactivates CXCL12[76,91]. However, the formation is no longer thought to be mainly the result of osteoclast activation, but rather to the loss of supporting cells, such as osteomacs and macrophages[65]. There is evidence that after administration of G-CSF, osteomacs leave the endosteal surface concurrent with endosteal osteoblast depletion[65]. (3) The third assumes a role of CD68/CD169 macrophages. The depletion of CD68/CD169+ macrophages seems to initiate a decreased expression of factors required for HSC retention (CXCL12), by selective downregulation of nestin+ mesenchymal stem cells (MSCs), as has been mentioned earlier[64,65]. That ultimately causes mobilization of HSCs into the PB. (4) The fourth involves complement activation. Activation of the complement cascade and thrombolytic pathway plays also a major role because of the release of sphingosine-1-phosphate (S1P) into the circulation by red blood cells, endothelial cells, and activated platelets. S1P is a strong chemoattractant of HSCs, creating an enabling environment for proliferation in the plasma[92,93]. S1P increases in blood and decreases in BM during mobilization, inhibiting SDF1 through the p38/Akt/mTOR pathway[92]. Both SDF1 and S1P are regulated by specificity protein (SP)-1, which it is thought to maintain a balance of their antagonistic effects. Several studies also suggest a role of the C5a complement component in mobilization, probably by neutrophil stimulation and the subsequent increase of MMP9 and decrease of CXCR4 expression. That is supported by the observation that C5-deficient mice respond poorly to G-CSF mobilization[94]. On the other hand, C3a expression promotes the chemotaxis of HSCs by CXCL12[94]. And (5) The fifth includes a role of the sympathetic nervous system. The role of the sympathetic nervous system (SNS) in G-CSF mobilization has been investigated. Sympathectomy or pharmacological innervation of the SNS[90] both lead to impaired mobilization in the mouse, and beta-2 (β2) agonist administration increases mobilization[90]. Another possible explanation is mobilization *via* nestin+ MSCs, which express many adhesion molecules, such as CXCL12, IL-17, and VCAM that are downregulated by β3 adrenoreceptor activation or G-CSF stimulation[95,96]. That observation explains why diabetes patients with impaired SNS function fail to mobilize adequate HSC numbers[97,98]. Summarizing, G-CSF upregulates CXCR4 in HPCs and decreases CXCL12 levels in the bone marrow relative to the blood and other tissues, establishing a chemo-attractive gradient that promotes migration of HSCs to the peripheral circulation.

Addition of chemotherapy as a mobilization strategy

For years there have been trials to establish a universal chemotherapeutic regimen, but without success because of uncontrolled or unknown variables. The optimal chemotherapeutic regimen for mobilization should have both antitumor activity and mobilization capacity[99]. Therefore, a chemotherapy regimen that is effective for the

underlying disease, either at relapse or first-line, in combination with G-CSF is used for PBSC mobilization. The main disadvantages are hematological toxicities, mobilization costs, and a rather unpredictable post-chemotherapy time for HSC harvest. Furthermore, it is essential to monitor the number of CD34 + cells in the PB every day. Considering the mechanism responsible for the effect of the chemotherapy regimens on bone marrow leading to stem cell mobilization, clear evidence exists only for cyclophosphamide (CY). Many studies have been conducted in humans, primates, and mice that showed release of active proteases in the bone marrow in response to G-CSF and CY[80,100]. The proteases cleave and inactivate many proteins that hold HSCs within the bone marrow stroma. CY increase the release of neutrophil proteases in the BM, with cleavage of VCAM-1 and decreased SDF-1a concentration in the BM. Winkler *et al*[101] demonstrated that CY induced a major reduction in SD-F1a mRNA ex-pression that promoted HSC mobilization without impairment of kit-ligand expression, indicating maintenance of niche functions and rapid recovery afterward. In addition, they observed a reduction in endosteal osteoblasts, bone formation, and F4/80+ osteomacs, while osteoid remained on the endosteum despite the absence of osteoblasts.

One of the often administered regimens is an intermediate dose of CY at 2-4.5 g/m^2, whereas high doses at 7 g/m^2 have been used as well, followed by the administration of G-CSF at a dose of 5-10 μg/kg/d[102]. Others used etoposide in combination with CY and/or cisplatin or added paclitaxel and concluded that the regimens were more effective for stem cell mobilization than CY alone. Moreover, Weaver *et al*[103] in 1998, used taxanes, either paclitaxel or docetaxel, in combination with CY, followed by G-CSF, and observed more efficient mobilization, almost three times more efficient than CY + G-CSF alone in patients with metastatic breast cancer[103].

The most frequently used regimen in patients with GCTs is paclitaxel at 200 mg/m^2 on day 1 plus ifosfamide at 2 g/m^2/d on days 1-3 (TI) supported with G-CSF at 10 μg/kg/d, starting on day 4[104,105]. TI was shown by Rick *et al*[104] more efficient than TI with the addition of cisplatin; *i.e.* the TIP regimen. An interesting mobilization regimen was used in the TAXIF study, wherein the epirubicin was added to paclitaxel. Despite the different chemotherapy mobilization regimens that have been used, the most commonly applied are TI or TIP, as was shown in a retrospective study by Hamid *et al*[106] (see also Table 1 for detailed references to the studies).

REMOBILIZATION STRATEGIES

Dose escalation of cytokines

Higher doses of G-CSF agents have been suggested as a strategy to improve mobilization and peripheral stem cell collection, but the evidence is conflicting. Some studies found no significant difference when a dose of 5 μg/kg/d was administered compared with the most broadly applied doses of 10 μg/kg[107,108]. Similarly, twice daily administrations did not demonstrate improved stem cell yields[109]. However a number of studies conducted in hematologic patients, provided compelling evidence that higher doses improved mobilization.

Structural modifications to improve poor physicochemical properties

Lenograstim: Lenograstim, a glycosylated form of G-CSF, also widely used for HSC transplantation, was hypothesized to induce increased mobilization compared to conventional G-CSF agents. In fact, it was proposed that its unique structure and glycosylation pattern provided protection against elastase-dependent inactivation, and could thereby lead to prolonged activity and increased mobilization[110,111]. Several studies though did not find any differences on HSC mobilization with collection results and patient outcomes comparable to conventional G-CSF-mobilized patients. Therefore, data on its efficacy remains to date both limited and inconclusive[112-114].

Pegfilgrastim: Pegfilgrastim is a pegylated form of G-CSF with long half-life characteristics because of its significantly reduced renal excretion[115]. It promotes stem cell mobilization with a single dose administration, as opposed to the daily injections of the regular short half-life G-CSF[116,117]. The results of recent studies have been controversial, as a number of them supported a significant increase in peripheral stem cells collected, while others found no difference in terms of stem cell mobilization, when a double dose of 12 mg-compared to the 6mg dose after conventional chemotherapy-was administered[118].

Table 1 Clinical studies applying various hematopoietic stem cell mobilization chemotherapy + granulocyte colony-stimulating factor protocols in patients with relapsed/refractory germ cell tumors

Ref.	Number of patients	Successful mobilization	Mobilization regimen
Fruehauf *et al*[149] 1995 (prospective analysis)	15	Median BM 31.49 × 10^6/kg PB 0.46 × 10^6/kg 100%	Cisplatin 100 mg/m^2 etoposide 75 mg/m^2 ifosfamide 2 g/m^2 + G-CSF
Tada *et al*[150] 1999 (retrospective analysis)	6	2.5 × 10^8/kg 100%	Cisplatin 200 mg/m^2 ifosfamide 4 g/m^2 etoposide 100 mg/m^2 d1-d3 + G-CSF
Rodenhuis *et al*[151] 1999 (multicenter prospective phase II)	35	10.3 × 10^6/kg 100%	Cisplatin 200 mg/m^2 ifosfamide 4 g/m^2 etoposide 100 mg/m^2 d1-d3 + G-CSF
Lotz *et al*[152] 2005 TAXIF 2005 (retrospective analysis)	45	9 × 10^6/kg (for 3 HDCT) 100%	Epirubicin 120 mg/m^2 - paclitaxel 200 mg/m^2 + G-CSF
Argawal *et al*[102] 2009 (retrospective analysis)	37	3-6 × 10^6/kg 100%	ifosfamide 2-4.5 g/m^2 + G-CSF
Feldman *et al*[153] 2010 (prospective phase I/II)	107	> 2 × 10^6/kg 100%	TI: paclitaxel 200 mg/m^2 d1 ifosfamide 2 g/m^2 d1-d3 + G-CSF
Haugnes *et al*[154] 2012 (prospective analysis)	882	> 2 × 10^6/kg 100%	BEP-ifosfamide + G-CSF
Mohr *et al*[155] 2012 (retrospective analysis)	44	> 4 × 10^6/kg 100%	PEI (cisplatin, etoposide, ifosfamide) + G-CSF Plerixafor in poor mobilizers
Necchi *et al*[156] 2015 (review)	42	> 2 × 10^6/kg 100%	BEP + G-CSF
Moeung *et al*[157] 2017 (pharmacokinetic phase II study)	89	> 9 × 10^6/kg (for 3 HDCT) (1-2 cycles) 100%	TI: paclitaxel, ifosfamide + G-CSF
Hamid *et al*[106] 2018 (retrospective analysis)	35	10/35 plerixafor + G-CSF 95%	TI: paclitaxel, ifosfamide or TIP
Argawal *et al*[158] 2019 (retrospective analysis)	321	172 allogeneic 95% 149 autologous 73% 77/149 without plerixafor → 64% success 72/149 with plerixafor → 82% success	G-CSF ± Plerixafor
Yildiz *et al*[159] 2020 (retrospective analysis)	50	> 2 × 10^6/kg 100%	TIP + G-CSF
Ussowicz *et al*[160] 2020 (retrospective analysis)	18 (children)	Median: 4.56 × 10^6/kg 100%	Cyclophosphamide 4 g/m^2 + G-CSF
Chevreau *et al*[161] 2020 (multicenter prospective phase II)	89	> 9 × 10^6/kg (for 3 HDCT) 100%	TI: paclitaxel, ifosfamide + G-CSF

G-CSF: Granulocyte colony-stimulating factor; HDCT: High-dose chemotherapy; TIP: Paclitaxel (Taxol)-ifosfamide-cisplatin.

Addition of mobilizing agents affecting a different pathophysiological pathway in order to improve peripheral stem cell collection

Ancestim: Ancestim is a recombinant human SCF that, through its binding to the c-kit receptor on HSCs, modulates their proliferation and adhesion, and has shown promising synergy in HSC mobilization when combined with G-CSF[119,120]. Limited efficacy when administered alone has also been noted[119]. Unfortunately, data available from recent studies did not confirm the efficiency in enhancing chemotherapy or growth factor-induced PBSC mobilization in patients with a prior insufficient PBSC collection, thus, limiting its further application[121].

GM-CSF: GM-CSF and its synergistic effect when combined with chemotherapy are no longer in use because the superiority of G-CSF in terms of mobilization and safety profile has been proved in a number of studies (*e.g.*, faster neutrophil recovery and fewer transfusions required)[122,123]. GM-CSF is sometimes used in combination with G-CSF in patients who failed an initial mobilization attempt, as a second or even as a third agent[124], despite the fact that several studies reported that the association of the two cytokines was not superior to G-CSF alone[125].

Plerixafor (Mozobil): Briefly, plerixafor was first studied as an agent against HIV [126]. During those clinical trials, neutrophilia was observed that sparked numerous studies[127]. In December 2008, plerixafor was approved by the Federal Drug Administration for use with G-CSF for HSC mobilization and collection and subsequent

ASCT in patients with non-Hodgkin lymphoma (NHL) and multiple myeloma (MM), who had failed prior mobilization with G-CSF alone or chemotherapy + G-CSF (plerixafor: AMD3100). The first report of the use of plerixafor in heavily pretreated, refractory and relapsed patients with GCTs was by Kobold *et al*[128]. Plerixafor was given subcutaneously in combination with G-CSF at a dose of 240 μg/kg after at least 4 d of G-CSF, which was given at the standard dose of 10 μg/kg/d. Plerixafor was administered 6 to 11 h before apheresis when a PB CD34+ count higher than 10/μL was achieved. The combination was successful, and allowed collection of sufficient numbers of CD34+ cells in 67% of the patients who failed prior mobilization with chemotherapy and G-CSF[128].

Despite the fact that the efficacy of plerixafor as a stem cell mobilization agent in patients with GCTs undergoing HDCT and ASCT has been reported in a number of small patient series and case studies, its use has not yet been approved, because of the lack of prospective studies. Thus, the indications for the use of plerixafor as a mobilization agent in patients with relapsed/refractory GCTs are not yet clear and rely on the opinions of the authors who published the studies (see Table 2 for details).

Structure and mechanism of action are as follows. Plerixafor (or AMD3100) is a bicyclam derivative that reversibly competes with and inhibits SDF-1a binding to CXCR4. CXCR4 is expressed on many cell types including white blood cells, epithelial, endothelial cells, and HPCs. It plays a critical role in the homing and trafficking of HPCs, as well as their retention and maintenance in the bone marrow niche. CXCR4 is a member of one of the two major families of chemokines. Chemokines are defined by the number and spacing of cysteine residues at the N-terminal end of the protein. CC cytokines have two cysteine residues that are adjacent; in CXC cytokines they separated by one amino-acid residue[129]. CXCR4 ligand, the chemokine SDF-1a (CXCL12), is produced by bone marrow stromal cells including osteoblasts, endothelial cells, and adventitial cells. Plerixafor was shown to directly inhibit SDF-1a ligand binding, SDF-1 mediated G-protein activation, calcium flux, and receptor internalization[130]. In another study, Lee *et al*[131] described the activation of phosphorylation of MAPK-p42/44 in granulocytes and monocytes by plerixafor, which induced the secretion of several proteases from the cells and enhanced the cleavage and activation of C5 in plasma. The C5 cleavage fragments (C5a and desArgC5a) play a critical role, as mentioned earlier, in the egress of HSCs. Granulocytes, stimulated and chemo-attracted by these fragments, enhance secretion of proteolytic enzymes that perturb HSCs retention signals and help HSCs to move through the endothelial barrier[131].

A possible mechanism for plerixafor-stimulated HSCs mobilization was proposed by Dar *et al*[132], in which an increase in CXCL12 circulating in the plasma was observed after the administration of plerixafor. At the same time, CXCL12 levels in BM fluids were decreased. The changes correlated with an increase of circulating progenitor cells in the blood, suggesting that SDF-1 actively regulated the number of circulating progenitor cells. Furthermore, the plasma levels of S1P, a potent chemoattractant for hematopoietic progenitors, was increased following AMD3100 administration[132].

The pharmacokinetics of plerixafor after subcutaneous injection show a peak plasma concentration within 30-60 min. Up to 58% of plerixafor is bound to plasma proteins, and it is eliminated by the urinary route with a half-life of 4 h. Similar increases in HSC levels are observed after multiple daily injections, suggesting no cumulative drug effect after consecutive injections[37,38]. An interesting fact about the timing of plerixafor injection and the mobilization of CD34+ was reported by Lefrere *et al*[38]. They found that in good mobilizers, the PB CD34 + count remained high for at least 12 h after G-CSF plus plerixafor administration[38]. In contrast, in poor mobilizers, precise monitoring of the PB CD34+ cell count was required, because the peak CD34+ cell count occurred 6-9 h after plerixafor injection[38]. It is essential to emphasize the significant decrease in CD34+ count that was observed in the patients 8-12 h after the injection, in order to determine the optimal timing of apheresis[38]. Regarding adverse effects, plerixafor is well tolerated, with rare reports of severe side effects, such as hypotension, dizziness, and thrombocytopenia. The most commonly observed adverse effects are diarrhea, nausea, and skin erythema at the injection site [38].

Future novel approaches: Most novel HSC mobilizing agents are initially tested in MM and NHL patients, and ASCT candidates. Successful application in that setting allows further testing in patients with relapsed/refractory GCTs and other solid tumors where HDCT and autografting are indicated at some point during the disease course. CXCR4 antagonists like plerixafor, emerged as potent agents to rescue "hard-

Table 2 Clinical studies applying plerixafor with granulocyte colony-stimulating factor ± chemotherapy for hematopoietic stem cells mobilization in patients with relapsed/refractory germ cell tumors

Ref.	Number of patients participating	Successful mobilization rates on previously failed chemotherapy + G-SCF driven mobilization (> 2 × 10^6)	Mobilization techniques
Kobold *et al*[128] 2011 (Retrospective analysis)	6	66.67% (4)	Chemo + G-CSF failed
			Plerixafor + G-CSF
Horwitz *et al*[162] 2012 (Retrospective analysis)	21	76% (17)	Chemo + G-CSF failed
			Plerixafor + G-CSF
Worel *et al*[163] 2012 (Retrospective analysis)	11	91% (10)	Plerixafor + G-CSF
Garcia-Escobar *et al*[164] 2014 (Case series)	5	80% (4)	Chemo + G-CSF failed
			Plerixafor + G-CSF
Kosmas *et al*[165] 2014 (Pilot study)	14 (3)	100% (3)	Chemo + G-CSF failed
			Chemo + Plerixafor + G-CSF
O'Hara *et al*[166] 2014 (Retrospective analysis)	9 (3)	100% (3)	Plerixafor + G-CSF

Related case studies: Saure *et al*[167], 2010; Tuffaha and Adel-Rahman[168], 2011; De Blasio *et al*[169], 2013; Miltiadous *et al*[170], 2017. G-CSF: Granulocyte colony-stimulating factor.

to-mobilize" patients with MM, NHL, GCTs, and some rare solid tumors. Research in that area has expanded with the development of novel CXCR4 inhibitors, such as motixafortide (BL-8040) and BKT140 (4F-benzoyl-TN14003), a 14-residue biostable synthetic peptide that binds CXCR4 with much greater affinity than plerixafor (84 nmol/L *vs* 4 nmol/L). An interim analysis of the phase 3 GENESIS trial of motixafortide *vs* placebo, both with G-CSF, for HSC mobilization in MM demonstrated an almost 4.9-fold increased efficacy in obtaining the primary endpoint of a target of 6.0 × 10^6 CD34+ cells/kg with up to two apheresis sessions and that 5.6-fold more patients achieved that target with one apheresis. Moreover, the motixafortide arm allowed 88.3% of patients to proceed to transplant, as opposed to 10.8% in the placebo arm [133]. Another peptide CXCR4 antagonist, a clinical stage compound balixafortide (POL6326) was evaluated in healthy volunteers and proved to be safe, well tolerated, and induced effective mobilization of HSCs at doses ≥ 1500 μg/kg and was predicted to yield an adequate collection of 4 × 10^6 CD34+ cells/kg in a single apheresis[134].

Another area of interest in HSC mobilization is the role of the sphingosine-1-phosphate/S1P receptor 1 (S1P/S1P1) axis, and studies in mice demonstrated an additional PB HSC mobilization benefit of S1P1 agonist (SEW2871) treatment in combination with a CXCR4 antagonist, but not human G-CSF[135]. However, that approach still remains experimental, with no apparent clinical testing so far.

Small molecule inhibitors of VLA-4 such as BIO5192 and monoclonal IgG4 antibodies (*e.g.*, natalizumab) bind to the a4 subunit of the a4β1 (VLA-4) integrin expressed on most leucocytes including CD34+ progenitor cells, inhibit the interaction of VLA4 primarily with VCAM-1 (CD106) on stromal cells, and secondarily with other ligands, including the segment-1 domain of fibronectin[136,137]. The interactions lead to increased HSCs in the blood. Therefore, their application has been proposed in patients with hematologic malignancies who are candidates for ASCT[138,139]. Unfortunately the clinical use of VLA-4 inhibitors is currently limited to multiple sclerosis and other inflammatory diseases.

Bortezomib (Velcade, PS-341) is a proteasome inhibitor that interferes with the activation of nuclear factor-kappa B (NFκB) by preventing proteasomal degradation of IκBa. VCAM-1 expression is upregulated by the VCAM-1 promoter. The latter is activated by binding to NFκB6. As proteasome inhibitors can indirectly inhibit transcription and expression of VCAM-1, and knowing the importance of the VCAM1-VLA4 interaction for HSC homing and mobilization, the application of proteasome

inhibitors as a mobilizer of HSC was proposed[140].

Hypoxia-inducible factor (HIF) prolyl hydroxylase (PHD) inhibitors, such as FG-4497, synergize with G-CSF and plerixafor to enhance mouse HSC mobilization. Deletion of the Hif1a gene weakens the effect[141]. A potential mechanism of FG-4497 proposed in recent studies includes stabilizing HIF-1a protein and increased VEGF-A secretion by BM macrophages[64,65]. FMS-like tyrosine kinase-3 Ligand (FLT3L) binds the FLT3 (CD135) receptor expressed on HSCs and induces proliferation, differentiation, development, and mobilization. Its efficacy has been shown either as a single agent, or in combination with other molecules mentioned above, such as IL-8 or G-CSF [142]. As chemokine-chemokine receptor axes are involved in retention of HSCs in the BM microenvironment, chemokine receptor agonists have been proposed as therapeutic agents to facilitate the mobilization process. The compounds include agonists of the CXCR4 receptor expressed on HSCs (*e.g.*, CTCE-0021 and ATI-2341)[143] or chemokines binding to chemokine receptors expressed on granulocytes and monocytes [*e.g.*, CXCL2, also known as the growth-related oncogene protein-beta (GRO-β) and its specific binding to the CXCR2 receptor; CCL3, also known as macrophage inflammatory protein-1α (MIP-1α); or CXCL8, also known as IL-8, could be used alone or in combination with other mobilizing agents like G-CSF or plerixafor (AMD3100)][144-146].

A novel mobilization strategy was developed and tested in mice through combined targeting of the chemokine receptor CXCR2 on granulocytes and VLA4 in HSCs. Treatment resulted in rapid and synergistic mobilization along with an enhanced recruitment of long-term repopulating of HSCs. That was achieved when a CXCR2 agonist, a truncated form of GRO-β; (tGRO-β) was administered in conjunction with a VLA4 inhibitor, leading to rapid and potent HSC mobilization, which represents an exciting potential strategy that warrants clinical development[147]. A G-CSF-free mobilization regimen using a tGRO-β compound, MGTA-145, which is a CXCR2 agonist, in combination with plerixafor was developed in the context of *in vivo* HSC transduction as a gene therapy approach in a mouse model of β-thalassemia[148]. The MGTA-145+plerixafor combination resulted in robust mobilization of HSCs. Importantly, compared with G-CSF + plerixafor, MGTA-145 + plerixafor led to significantly less leukocytosis and no elevation of serum interleukin-6 levels, and was thus likely to be less toxic[148]. However, the above regimen has not yet been tested for HSCs mobilization in neoplastic diseases. Therefore, evidence is accumulating that CXCR4 receptor agonists could be used with other agents as mobilizing drugs. In particular, they may provide an alternative for patients who are poor mobilizers.

CONCLUSION

Despite the fact that GCTs are currently considered as curable tumors, almost 30% of patients presenting with metastatic disease at diagnosis are likely to experience disease progression at some point. The use of HDCT and ASCT has been established as a salvage therapeutic option, but a number of patients fail to mobilize with conventional strategies. Such poor mobilizers endanger the safety of the procedure. Along with conventional mobilization strategies, such as G-CSF and chemo-mobilization, the use of newer mobilizing agents like plerixafor has emerged with promising results for this group of patients.

Algorithms to improve the efficiency of HSC mobilization, for example "just in time" and preemptive, aim to minimize failures, obtain the desired CD34+ HSCs dose for one or more transplants with the least apheresis sessions, and thus reduce overall healthcare costs, are urgently required. As novel HSC mobilizing agents are initially tested in preclinical experimental models and hematologic malignancies, such as NHL and MM, their application in solid tumors, candidates for ASCT, and in particular GCTs, is lagging behind.

Two axes responsible for HSC retention in the BM stroma that have been explored are the CXCR4-CXCL12 (SDF-1) and the VLA4 (α4/β1)-VCAM1 pathways. Novel inhibitors of those interactions have been evaluated, either alone or in combination with G-CSF, or with GRO-β/CXCR2 axis co-stimulation. Nevertheless, as studies in this area are limited, future investigation should concentrate on finding new agents or establishing proper mobilization algorithms to achieve an adequate CD34+ dose required for a successful ASCT.

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REVIEW

Re-irradiation for high-grade gliomas: Has anything changed?

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Abstract

Optimal management after recurrence or progression of high-grade gliomas is still undefined and remains a challenge for neuro-oncology multidisciplinary teams. Improved radiation therapy techniques, new imaging methods, published experience, and a better radiobiological knowledge of brain tissue have positioned re-irradiation (re-RT) as an option for many of these patients. Decisions must be individualized, taking into account the pattern of relapse, previous treatment, and functional status, as well as the patient's preferences and expected quality of life. Many questions remain unanswered with respect to re-RT: Who is the most appropriate candidate, which dose and fractionation are most effective, how to define the target volume, which imaging technique is best for planning, and what is the optimal timing? This review will focus on describing the most relevant studies that include re-RT as salvage therapy, with the aim of simplifying decision-making and designing the best available therapeutic strategy.

Key Words: Re-irradiation; Recurrent glioma; High-grade gliomas; Glioblastoma; Radiosurgery; Stereotactic radiotherapy

Core Tip: The optimal management after recurrence or progression of high-grade gliomas is still undefined. Improved radiation therapy techniques, new imaging methods, published experience, as well as better radiobiological knowledge of the brain tissue have positioned re-irradiation as a valid alternative for many of these patients. Many questions remain unanswered. This review will focus on describing the most

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INTRODUCTION

High-grade gliomas (HGG) are the most common primary malignant brain neoplasm in adults[1]. The most frequent type, glioblastoma multiforme (GBM), has an incidence of 3 cases/100000 inhabitants[2]. Its treatment is a macroscopically complete tumor resection, whenever possible, followed by external beam radiotherapy (60 Gy in 2 Gy/fr) with concurrent temozolomide (TMZ) and adjuvant TMZ until the completion of six cycles[3]. Nevertheless, approximately 40% of World Health Organization (WHO) grade III gliomas (anaplastic astrocytoma) and 90% of grade IV gliomas (GBM) progress within 2 years. The main site of relapse is in or near the tumor bed[3-5].

With standard treatment, median overall survival (mOS) for GBM is approximately 14.6 mo, and median progression-free survival (mPFS) is 6.9 mo[6]. This tumor has a poor prognosis and is very aggressive and fast-growing. The high rate of local failure suggests secondary therapeutic options for local salvage should be considered.

The first issue during the diagnostic-therapeutic approach is to confirm that we are dealing with true tumor progression. The phenomena of "pseudoprogression", described in 20%-30% of patients who have received radiochemotherapy and possible radionecrosis (RN), associated or not with tumor, may hinder or delay diagnosis[7]. The Response Assessment in Neuro-Oncology working group criteria[8] for HGG categorization has certain limitations.

Optimal management after recurrence or local progression remains to be defined. It has mostly been established by retrospective studies lacking a quality of life (QoL) evaluation. Established salvage treatment options include a second surgery (re-S), re-irradiation (re-RT), systemic treatment, or some combination thereof[9]. The addition of the "tumor treating field therapy" approach (alternating electrical fields that exert biophysical force on charged and polarizable molecules known as dipoles) has been found to extend survival for patients with newly diagnosed and recurrent GBM (rGBM)[10].

These suboptimal results have motivated multiple lines of research investigating new therapeutic approaches such as the addition of molecular targeted agents, immune checkpoint blockade, vaccines, viral therapy, or other irradiation modalities[11-14].

Current therapeutic approaches, including the radiation therapy techniques and parameters, are very diverse. Thus, a survey of expert radiation oncologists showed high variability, reflecting the scarcity of high-quality prospective data for decision-making[15]. Multiple questions remain unanswered with respect to re-RT: Who is the most appropriate candidate, which dose and fractionation are most effective, how to define the target volume, and which imaging technique is best for planning, as well as the optimal timing? This review will focus on describing the most relevant studies that include re-RT as salvage therapy, with the aim of simplifying de-cision making and designing the best available therapeutic strategy.

RE-RT IN THE THERAPEUTIC STRATEGY

At present, any ablative treatment option offered to a selected patient with local failure is still palliative and has associated side effects that must be considered. The choice is complex, and the criteria are poorly defined. Decisions must be individualized, taking into account the pattern of relapse, previous treatment, and functional status, as well as the patient's preferences and expected QoL.

For patients with low functional status, unable to walk and totally dependent for daily activities, the best supportive care should be considered.

Historically, the fear of exceeding the dose tolerance of healthy brain tissue, and therefore the risk of severe side effects, kept radiation oncologists from considering re-RT with ablative doses. Thus, the most offered treatment has been systemic [chemotherapy/bevacizumab (BEV)], with a mOS of 6-9 mo, without a clear advantage of any drug or therapeutic scheme among those used[16,17]. Clearly this is the best strategy for patients with widespread or multifocal disease. However, in the case of a focal relapse, if the patient has favorable clinical criteria, the current trend is to consider a second local treatment such as re-S, re-RT, or both with or without systemic treatment.

The level of evidence supporting this approach is low, probably because the high failure rates (recurrence or progression) of these second treatments make it difficult to compare the different strategies.

Objective parameters are needed to simplify therapeutic decision-making. Scoccianti *et al*[18], based on a review of the literature, recommend the first algorithm to aid decision-making in daily practice between surgical salvage or re-RT. They consider local treatment for focal relapses in patients with life expectancy > 3 mo. The choice of re-S or re-RT depends on prognostic factors and the expected toxicity of each therapeutic option. The results of combined treatment are encouraging, and the tendency is to recommend it. The therapeutic decision should be interdisciplinary and requires expert neurosurgeons and radiation oncologists. Ultimately, the final decision should be agreed upon with the patient after discussion of the risks and benefits of the available therapeutic options.

RESULTS

Re-resection

A minority of patients (20%-30%) are considered eligible for re-S[19], with a higher morbidity-mortality than before initial resection. After re-S, overall survival from re-RT ranges from 4.9[20] to 13.5 mo[21] and PFS from re-RT from 1.9[22] to 8.3 mo[23]. These results are from retrospective, not comparative series. There is no evidence to suggest that these results are better than can be expected with radiation and/or chemotherapy alone[24,25]. The meta-analysis of Lu *et al*[26] suggests that re-S of rGBM in select patients confers a significant, prognostic OS advantage independent of other prognostic factors, and a cohort from The Director Trial[27] found that surgery at first recurrence of GBM improved outcome if complete resection of contrast-enhancing tumor was achieved. Preoperative and postoperative Karnofsky performance status (KPS), extent of surgery of first re-S, and chemotherapy after first re-S have been identified as the factors that have the greatest impact on survival[25].

Due to the absence of comparative studies, the role of re-S in rGBM is not yet established. The Randomized Controlled Comparative Phase II Trial on Surgery for Glioblastoma Recurrence trial comparing re-S of recurrence plus second-line treatment, *vs* second-line treatment without re-S, will quantify the contribution of re-S for rGBM.

Re-RT

Based mostly on retrospective series, selected patients with small recurrent tumors and a good performance status may benefit from re-RT using modern high-precision techniques[28-31]. Prospective studies are very scarce, therefore the exact contribution of re-RT is uncertain.

The tumoricidal dose to be administered is limited by the possibility of generating severe side effects, given that most patients have already received doses in the maximum tolerance range at their first irradiation. Re-RT at the therapeutic doses used at diagnosis (60 Gy) is not recommended.

Potential benefits of re-RT include palliation by reducing corticosteroid use, improving neurologic symptoms, and, in selected patients, increasing PFS and possibly overall survival.

There are three most commonly used external radiation therapy techniques that, depending on the fractionation applied, the treatment volume, and the technology used we refer to as: Stereotactic radiosurgery (SRS), hypofractionated stereotactic radiotherapy (HFSRT), and conventionally fractionated external radiotherapy (CFRT). We also have results with intraoperative techniques[32]. The promising results of particle irradiation are described in the section on new irradiation strategies. The

choice of technique, in addition to its geographical availability, depends on the size of the recurrence and consequently of the planning target volume (PTV) generated.

Unfortunately, the lack of comparative trials does not allow their results to be compared. However, even in the absence of randomized data, there is a tendency to use hypofractionated or SRS schemes for small volumes, assuming a slightly higher risk of RN.

Kazmi *et al*[33] published the first meta-analysis with the results of re-RT in rGBM. They included 50 studies with a total of 2095 patients. Overall survival from re-RT and PFS from re-RT at 6 mo were 73% and 43%, respectively, and at 12 mo were 36% and 17%. They found better PFS at 6 mo with SRS and with short fractionation schedules (≤ 5 fractions), probably due to the lower tumor volume.

SRS as salvage treatment

Table 1 describes a selection of series published since 2005. They are characterized by: Including mostly GBM, a single dose of 12-18 Gy, a median volume of around 10 mL, and a time from the first radiation treatment of between 8.8 mo and 13.8 mo. The Kong series[34] is the largest and the only prospective series. The mOS for GBM is between 7.5 mo and 13 mo, while the range for mPFS, in those series that report it, is between 3.6 mo and 7 mo. Severe toxicity is not reported, except for RN, which in a couple of series is 24%-31% by radiological imaging. These data suggest that patients with small volumes can be safely treated with SRS.

Fractionated stereotactic radiotherapy as salvage treatment

Hypofractionated schemes have been used mainly in larger recurrent HGG (rHGG). A selection of studies published in 2000 or later, including several prospective series, are presented in Table 2. Some contain anaplastic and low-grade gliomas. The median dose and fractionation used are highly variable, between 25 and 35 Gy (3-7 Gy/fr), with an equivalent dose at 2 Gy (EQD2) range of 37.5-78.7 Gy. The largest series is Fogh *et al*[29] with 147 patients, of which 42 had anaplastic astrocytomas, with an average dose of 35 Gy (3.5 Gy/fr) and a mOS of 11 mo for rGBM. Severe toxicity is also highly variable, with some series reporting none and others as much as 10.5% and a percentage of radiological RN between 6%-11%.

A recent study, in a large and heterogeneous series of 198 patients with rHGG, reports a mOS of 7 mo (6 mo for GBM and 14 mo for grade III gliomas) with good tolerance. The most common fractionation schedules were 41.8 Gy-49.4 Gy/3.8 Gy/fr [35].

The main study with CFRT is by Combs *et al*[36]. They analyzed 59 patients with rGBM treated with 36 Gy/2Gy/fr, achieving an mOS of 8 mo, with only 1.7% of histologically confirmed RN despite a large median tumor volume (49.3 mL). This indicates that it may be an adequate schedule in larger lesions.

Several retrospective papers have compared the different techniques (SRS, HFSRS, CFRT), reporting similar results between them, with mOS of 9.7-11 mo[37,38].

There are very few prospective studies on the efficacy of re-RT *vs* systemic treatment alone. RTOG 0525[39] has reported mOS of 8.2 mo with re-RT, 10.5 mo with chemotherapy, and 11.3 mo with radiochemotherapy. Patients who only received best supportive care had an mOS of 4.8 mo, probably selected for worse overall status. Available data in rGBM generally suggest that re-RT modestly improves PFS compared with systemic treatment alone, but OS is similar[40].

Re-RT of larger volumes

The main hurdle for re-RT of voluminous relapses has been the risk of RN. Most re-RT studies describe a PTV < 40 mL[41,42]. The available evidence for large volume lesions is sparse and few studies include a median PTV greater than 75 mL. Two authors report the largest series to date. The study by Scholtyssek *et al*[43], with a median PTV of 110.4 mL and doses of 36 Gy (30 Gy-40.05 Gy) at 2-5 Gy/fr, did not describe severe toxicity or RN. Chan *et al*[44], with a median PTV of 145.3 mL and dose of 35 Gy/15 fr, in 67 patients, reported 4 cases of radiological RN. The mOS reported in these series were 7.7 and 7.8 mo, in the same range as reported in studies with small treatment volumes. We can conclude that re-RT of large volume disease is feasible, provided that the doses administered are appropriate.

Re-RT with concurrent systemic treatment

Two drugs (TMZ, BEV) are mainly used. Although they have been shown to be safe combinations, their benefit has yet to be demonstrated.

Table 1 Summary of selected publications reporting radiosurgery as salvage treatment in recurrent high-grade gliomas

Ref.	Study type	No. patients	Histology	Re-irradiation		Median interval	Median tumor volume	Median PFS2	Median OS2	Severe toxicity	Radionecrosis
				Total dose, median	Dose/fr, median						
Combs *et al* [28], 2005	R	32	All GBM	15 Gy	63.8 Gy	10 mo	10 mL	7 mo	10 mo	0%	0%
Hsieh *et al* [104], 2005	R	26	All GBM	12 Gy	42 Gy	NR	21.6 mL	NR	10 mo	NR	31.3% by image
Kong *et al* [34], 2008	P	114	65 GBM, 49 G3G	16 Gy	72 Gy	NR	10.6 mL	4.6 mo (GBM), 8.6 mo (G3G)	13 mo (GBM), 26 mo (G3G)	0%	24.4% by image
Patel *et al* [68], 2009	R	26	All GBM	18 Gy	90 Gy	12.5 mo	10.4 mL	NR	8.4 mo	Limited toxicity	NR
Skeie *et al* [30], 2012	R	51	All GBM	12.2 Gy	43.3 Gy	11 mo	12.4 mL	6 mo	12 mo	0%	0%
Martínez-Carrillo *et al*[31], 2014	R	87	46 GBM, 41 G3G	18 Gy	90 Gy	13.8 mo	8.7 mL	NR	7.5 mo (GBM); 17 mo (G3G)	0%	0%
Kim *et al* [105], 2015	R	29	All GBM	15 Gy	63.8 Gy	8.8 mo	11 mL	3.6 mo	9.2 mo	NR	NR

α/β = 2; EQD2: Equivalent dose at 2 Gy fractions; G3G: Grade III glioma; GBM: Glioblastoma; NR: Not reported; OS2: Overall survival from re-irradiation; P: Prospective; PFS2: Progression free survival from re-irradiation; R: Retrospective.

Re-RT with TMZ: Table 3 summarizes the main results. The techniques used have been HFSRS or CFRT. Hematologic ≥ grade 3 toxicity of up to > 40% has been described. RN has been reported, either radiological (7%-8%)[45,46] or histopathological in 4.3%[47]. The mOS for GBM ranges from 9.7-14 mo[45,48] and mPFS between 4-7 mo[46,47], results reported without the combination of TMZ. However, in the Grosu *et al*[49] and Conti *et al*[47] series, patients receiving TMZ had higher mOS.

Overall, concurrent approaches with TMZ do not appear to improve re-RT outcomes and may carry increased risk of toxicity. However, these findings need to be confirmed in prospective series.

Re-RT with BEV: The association of BEV to treatment with first line radiochemotherapy did not demonstrate a benefit in OS in two phase III trials[50,51]. In recurrences, the role of concurrent BEV with re-RT is still not well defined, but several studies have confirmed the safety of this combination with reasonable survival results [52-57]. Table 4 summarizes the main results. The mOS ranges between 9.3-12.2 mo for rGBM, with mPFS between 5.2 and 6.8 mo. This combination has been shown to decrease the risk of RN, especially for re-RT of larger volumes[44,58]. The percentage of symptomatic RN/symptomatic edema, defined as the need for corticosteroids > 6 wk after re-RT, was lower with the BEV combination (21.8% *vs* 37.8%, $P = 0.025$), with these differences increasing at 1 year (23.9% *vs* 54.1%, $P = 0.013$).

The highly anticipated results from the NRG Oncology/RTOG 1205 phase II clinical trial (NCT01730950) are expected in 2023. It randomizes patients with recurrence to BEV alone or BEV with concurrent re-RT (35 Gy in 10 fractions for tumors smaller than 5 cm). Preliminary results of this study have confirmed the safety of the BEV-Re-HFSRT combination and that it provides a benefit in PFS at 6 mo, even without a benefit in mOS, as observed in first line.

Re-RT after progression to BEV

Recently, a new scenario of re-RT has been explored, after progression to BEV. Several groups have published data on this approach, showing an mOS of 5.4 mo[59] and 4.8 mo[39]. The combination of minocycline, BEV, and fractionated re-RT after progression to BEV has been investigated in a phase I trial[60]. PFS3 was 64.6%, and mOS was 6.4 mo. This study adds a prospective trial to the literature showing that re-RT of HGG after BEV failure can be performed with acceptable tolerability. Another recently published phase I trial included 32 patients with rHGG and the combination of

Table 2 Summary of selected publications reporting hypofractionated stereotactic radiosurgery as salvage treatment in recurrent gliomas

Ref.	Study type	No. patients	Histology	Re-irradiation			Median interval	Median tumor volume	Median PFS2/actuarial PFS2	Median OS2	Severe toxicity	Radionecrosis
				Total dose, median	Dose/fr, median	EQD2						
Selch *et al*[106], 2000	R	21	15 GBM, 3 G3G, 2 G2G, 1 no biopsy	25 Gy	5 Gy	43.8 Gy	11 mo	12 mL	5 mo	6.7 mo	0%	0%
Vordermark *et al* [107], 2005	R	19	9 GBM, 10 G2G	30 Gy	5 Gy	52.5 Gy	19 mo	15 mL	4.9 mo, 4.6 mo (GBM)	9.3 mo, 7.9 mo (GBM)	10.5% other than necrosis	0%
Ernst-stecken *et al* [108], 2007	P	15	10 GBM, 3 G3G. 2 G2G	35 Gy	7 Gy	78.7 Gy	10 mo	22.4 mL	15 mo	12 mo	20% need to increase steroids dose without evidence of progressive disease	NR
Fokas *et al*[78], 2009	P	53	All GBM	30 Gy	3 Gy	37.5 Gy	NR	35 mL	22% at 12 mo	9 mo	0%	0%
Fogh *et al*[29], 2010	R	147	105 GBM, 42 G3G	35 Gy	3.5 Gy	48.1 Gy	8 mo	22 mL	NR	11 mo (GBM)	0.7% toxicity (severe headaches)	0%
Mckenzie *et al*[69], 2013	P	33	29 GBM, 4 G3G	30 Gy	5 Gy	52.5 Gy	NR	8.54 mL	62% at 6 mo	8.6 mo	9% toxicity other than necrosis	9% by image
Ogura *et al*[80], 2013	R	30	15 GBM, 9 G3G. 6 G2G	35 Gy	7 Gy	78.7 Gy	NR	9 mL	3 mo	10.2 mo	13.3% need to increase steroids dose without evidence of progressive disease	6.1% by image
Miwa *et al*[109], 2014	P	21	All GBM	30 Gy	5 Gy	52.5 Gy	12 mo	27.4 mL	NR	11 mo	4.8%	9.5%
Dincoglan *et al* [110], 2015	R	28	All GBM	25 Gy	5 Gy	43.8 Gy	11.2 mo	36.5 mL	5.8 mo	10.3 mo	0%	11% G2 by image

α/β = 2; EQD2: Equivalent dose at 2 Gy fractions; G: Grade; G2G: Grade II glioma; G3G: Grade III glioma; GBM: Glioblastoma; NR: Not reported; OS2: Overall survival from re-irradiation; P: Prospective; R: Retrospective; PFS2: Progression free survival from re-irradiation.

pembrolizumab, an anti-programmed cell death protein 1 (PD-1) monoclonal antibody, HFSRT, and BEV, with an mOS and mPFS of 13.4 mo and 7.9 mo, respectively. The authors concluded that this combination is safe and well tolerated, meriting further investigation[61].

Re-resection and re-radiation therapy

Straube *et al*[62] was the first author to suggest that this strategy could be beneficial, after concluding that the pattern of relapse in 26 patients with complete re-S was solely local in 70%. Based on this, and taking into account the maximal safe resection, several groups have demonstrated the value of additional re-RT with different techniques[38,

Table 3 Summary of selected publications reporting re-irradiation plus temozolomide as salvage treatment in recurrent high-grade gliomas

Ref.	Study type	No. patients	Histology	Re-irradiation			Median interval	Median tumor volume	Median PFS2/actuarial PFS2	Median OS2/actuarial OS2	Severe toxicity	Radionecrosis
				Total dose, median	Dose/fr, median	EQD2						
Grosu *et al*[49], 2005	P	44 (TMZ 29)	34 GBM, 2 Gliosarcomas, 8 G3G	30 Gy	5 Gy	52.5 Gy	16 mo	15 mL	NR	8 mo (11 mo RT + TMZ *vs* 6 mo without TMZ)	0%	0%
Combs *et al*[81], 2008	R	25	8 GBM, 10 G3G, 7 G2G	36 Gy	2 Gy	36 Gy	36 mo	50 mL	5 mo; 16% at 12 mo	8 mo; 25% at 12 mo	0%	NR
Minniti *et al*[45], 2011	R	36	All GBM	37.5 Gy	2.5 Gy	42.2 Gy	14 mo	13.1 mL	5 mo; 8% at 12 mo	9.7 mo; 33% at 12 mo	Thrombocytopenia G3: 2.8%	8% by image
Conti *et al*[47], 2012	R	23 (TMZ 12)	All GBM	20 Gy	10 Gy	60 Gy	7 mo	< 30 mL	7 mo (TMZ) *vs* 4 mo (no TMZ)	12 mo (TMZ) *vs* 7 mo (without TMZ)	≥ G3 hematological toxicity > 40%	4.3%
Minniti *et al*[46], 2013	R	54	38 GBM, 16 G3G	30 Gy	6 Gy	60 Gy	15.5 mo	9.8 mL	6 mo (4 mo GBM)	12.4 mo (11.4 mo GBM)	Thrombocytopenia G3: 3.7%, leukopenia G3: 3.7%	7% by image
Greenspoon *et al*[111], 2014	P	31	All GBM	30 Gy	5 Gy	52.5 Gy	At least 6 mo	12 mL	7 mo	9 mo	NR	G3: 9.6%, G4: 3.2%
Aktan *et al*[48], 2015	R	21 (17 TMZ)	18 GBM, 3 G3G	54 Gy	2 Gy	54 Gy	39.4 mo	Recurrent tumor size was median 5.5 cm	NR	18 mo (G3G) and 14.1 mo (GBM)	0%	0%

α/β = 2; EQD2: Equivalent dose at 2 Gy fractions; G2G: Grade II glioma; G3G: Grade III glioma; G: Grade; GBM: Glioblastoma; NR: Not reported; OS2: Overall survival from re-irradiation; P: Prospective; R: Retrospective; PFS2: Progression free survival from re-irradiation; TMZ: Temozolomide.

63-65].

Combs *et al*[63] published the first study after rHGG re-S followed by early re-RT. It included 108 patients, most of whom received 36 Gy at 2-3 Gy *per* fraction. The mOS was 12 mo, with no serious toxicity. In multivariate analysis, the extent of surgery, methylguanine-DNA methyltransferase (MGMT) methylation, interval time between first and second irradiation, and KPS were independent prognostic factors for OS. A subsequent study[64], with 25 interventional rGBM cases treated with HFSRS and simultaneous integrated boost (37.5 Gy and 45 Gy in 15 fractions), reported an mPFS of 13 mo and mOS of 16 mo, with better outcomes in smaller recurrences, without eloquent area involvement and in patients with a good general condition.

On multivariate analysis, the macroscopic tumor volume (GTV) ≥ 100 mL *vs* < 100 mL was confirmed as an independent prognostic factor affecting OS. Radiologically suspected RN was observed in 16 patients (64%) at a median of 9 mo after re-RT, and 8 patients developed grade 3 RN requiring hospitalization.

Table 4 Summary of selected publications reporting re-irradiation plus bevacizumab as salvage treatment in recurrent high-grade gliomas

Ref.	Study type	No. patients	Histology	Re-irradiation			Median interval	Median tumor volume	Median PFS2	Median OS2	Severe toxicity	Radionecrosis
				Total dose, median	Dose/fr, median	EQD2						
Gutin *et al* [56], 2009	P	25	20 GBM, 5 G3G	30 Gy	6 Gy	60 Gy	14.5 mo	34 mL	7.3 mo	12.5 mo	G3: 1 hemorrhage; G4: 3 (1 bowel perforation, 1 wound dehiscence and 1 GI bleed)	0%
Cuneo *et al* [54], 2012	R	63 (41 BEV)	49 GBM, 8 G3G, 6 prior G2G	15 Gy	15 Gy	63.8 Gy	21 mo	4.8 mL	GBM: 5.2 mo (BEV) *vs* 2.1 mo (without BEV). 6 mo whole series	GBM: 11.2 mo (BEV) *vs* 3.9 mo (without BEV). 10 mo whole series	11%	10%
Niyazi *et al* [52], 2012	R	30 (20 BEV)	22 GBM, 8 G3G	36 Gy	2 Gy	36 Gy	NR	NR	8 mo	Mean 12 mo	G3:1; G4: 1 wound dehiscence	0%
Shapiro *et al* [112], 2013	R	24	20 GBM, 1 G3G, 3 G2G	30 Gy	6 Gy	60 Gy	12.6 mo	35.3 mL	7.5 mo (6.8 mo GBM)	12.2 mo (whole series and GBM)	Toxicity BEV: G4: 12.5%	0%
Cabrera *et al* [113], 2013	P	15	8 GBM, 7 G3G	18 Gy. 25 Gy	18 Gy. 5 Gy	90 Gy. 43.8 Gy	20 mo	NR (< 5 cm)	3.9 mo	14.4 mo	G3:1	0%
Flieger *et al* [57], 2014	P	71 (57 BEV)	52 GBM, 19 G3G and G2G	36 Gy	2 Gy	36 Gy	NR	NR	5.6 mo (BEV) *vs* 2.5 mo (without BEV)	GBM: 9.3 mo (BEV) *vs* 6.1 mo (without BEV)	Toxicity BEV: G4: 5.3%	4.2% (BEV) by image or histologically

α/β = 2; BEV: Bevacizumab; EQD2: Equivalent dose at 2 Gy fractions; G: Grade; GBM: Glioblastoma; G2G: Grade II glioma; G3G: Grade III glioma; NR: Not reported; OS2: Overall survival from re-irradiation; P: Prospective; PFS2: Progression free survival from re-irradiation; R: Retrospective.

In the series by Chun *et al*[65] with 84 patients, the addition of radiation therapy (median dose of 45 Gy at 1.8 Gy/fr) to re-S was associated with a significant benefit in PFS, with mPFS for re-S being 3.5 mo and 9 mo for re-S plus re-RT. The benefit in OS was marginal, with an mOS of 12.7 mo with re-S *vs* 28.1 mo with re-S plus re-RT (P = 0.066). Three risk factors (age ≥ 50, WHO grade IV, and unmethylated promoter of MGMT) were significantly associated with poor OS in multivariate analysis. The authors established three categories of risk groups based on these factors. The benefit of re-RT in both OS and PFS was established in patients with two or more risk factors (intermediate and high risk groups). There was no radiological or pathological evidence of RN during or after re-RT.

Results of the GlioCAVE/NOA 17 trial (NCT02715297) should better determine the contribution of early adjuvant radiotherapy after re-S in rGBM. It is a prospective phase II study with a schedule of 46 Gy at 2 Gy/fr or 36 Gy at 3 Gy/fr, with PFS as the primary endpoint.

Prognostic scales for re-RT

The first scale to predict OS after re-RT published by Combs[66] derived from 233 patients with recurrent low- and HGG and included: WHO grade, age at the time of re-RT, and the time interval to re-RT. The same group published a modified version, the New Combs Score, which added other factors such as KPS, tumor volume, and re-S prior to re-RT[67]. This new revalidated scale[38,63] with a simple approach, is practical, useful, and widely used for decision making (Table 5).

Other reported prognostic features include the re-RT dose[31,57], use of salvage chemotherapy[43,57], extent of resection[28,43], MGMT promoter methylation status [46], and radiographic response[68-70]. However, how they should be quantified remains to be described.

Interestingly, Chapman *et al*[38], without finding an association of irradiation technique (SRS *vs* non SRS) or fractionation with survival, identified a threshold dose as a function of PTV size that should not be exceeded to minimize toxicity: 40 Gy Biological Equivalent Dose 10 for SRS (16 Gy in 1 fraction) and 45 Gy Biological Equivalent Dose 10 for non-SRS treatments (approximately 30 Gy in 5 fractions, 35 Gy in 10 fractions or 40 Gy in 20 fractions), from the same range as those identified in other series[29,57,71]. And, globally, it identifies a group of patients who can achieve an advantage in OS and PFS with re-RT, in particular young patients with good KPS, longer time interval from initial radiation to first progression, small recurrence volume, and an adequate re-RT dose.

RADIOTHERAPY SPECIFICATIONS

Treatment volumes

Definition of target volumes: The definition of re-RT target volumes should be conservative, minimizing the irradiation of healthy tissues to avoid severe toxicities (RN). It requires not only extreme precision and conformality during treatment but also precise images that identify the exact location of tumor tissues. Inaccuracies in tumor delineation may diminish any gain in local control achieved by dose escalation. One aspect to consider would be whether the relapse is located in the area of previous maximum dose or is marginal or remote from the first irradiation. In this case, and depending on the volume of the relapse, the dose prescription can be less conservative.

Several studies have shown that standard anatomic imaging modalities [computed tomography, magnetic resonance imaging (MRI)], while very accurate in visualizing normal anatomic structures, are limited in defining the exact extent of the tumor. Classically, volume delineation for irradiation is based on T1-weighted MRI with gadolinium. Contrast uptake is a consequence of blood-brain barrier disruption and does not necessarily reflect the actual tumor extent in gliomas. Macroscopic tumor masses far from the margins of contrast enhancement have been detected in surrounding edema and even in adjacent normal-appearing brain tissue[72-74]. Anti-angiogenic drugs may also condition contrast uptake, as they may initially have a stabilizing effect on the blood-brain barrier[75].

Multiple studies correlating imaging findings with histopathologic evaluation in surgically treated patients with HGG have indicated that molecular imaging with amino acid positron emission tomography (PET) is more specific and equally sensitive for tumor detection than MRI (T1 with gadolinium). Grosu *et al*[49] have postulated that target volumes for re-RT should be based on amino acid PET imaging in addition to MRI, to include the actual tumor dimension. Other imaging modalities have been used to delineate GTV, including spectroscopy MRI, perfusion-weighted imaging and diffusion-weighted imaging[76], 11C-methionine PET[49], and 18 F-dihydroxyphenylalanine PET[46]. However, there are no randomized trials that have evaluated the impact of molecular or functional imaging-based radiotherapy on the outcomes achieved.

The ongoing phase II GLIAA (NOA 10/ARO 2013-1) trial[77] is the first randomized study evaluating the impact of differences in planning volumes designed with molecular *vs* MRI imaging on PFS after re-RT in patients with rGBM. The limited availability of molecular and/or functional imaging equipment together with the lack of evidence of its superiority in the design of planning volumes conditions the continued use of MRI images for re-RT volume definition.

Volumes-exclusive radiotherapy: The definition of the target volume generally includes the GTV, defined as any contrast-enhancing lesion on T1-weighted MRI. In most studies, the clinical target volume (CTV) equals GTV[28,29,78]. Some papers add

Table 5 Scoring scheme and new prognostic groups of the "New Combs Score"

Prognostic factors	Prognostic value
Primary histology	
Glioblastoma, WHO IV	2
Anaplastic glioma, WHO III	1
Low-grade glioma, WHO I/II	0
Age	
≥ 50 yr	1
< 50 yr	0
Time between primary RT and re-RT	
≤ 12 meses	1
> 12 meses	0
Re-resection performed	
No	1
Yes	0
KPS	
< 80%	1
≥ 80%	0
Tumor volume (PTV)	
> 47 mL	1
≤ 47 mL	0
Scoring group	Scoring value/mOS
a	0–1/19.5 mo
b	2–3/11.3 mo
c	4–5/8.1 mo
d	6–7/5.5 mo

KPS: Karnofsky performance status; mOS: Median overall survival; PTV: Planning target volume; RT: Irradiation; WHO: World Health Organization.

a CTV to include the peritumoral edema visualized in the fluid-attenuated inversion recovery sequence of the MRI, since it is known that tumor cells can be found in this location[79,80]. Subsequently, a margin usually ≤ 5 mm is added for PTV expansion [45,49,78], although some authors include up to 1 cm[57,81].

Volumes-adjuvant radiotherapy: For re-S patients, Straube *et al*[62] proposed a GTV including the resection cavity and contrast enhancement areas, with a margin of 5-10 mm to generate the CTV and 1-3 mm to create the PTV. The GLIOCAVE-NOA 17 study[82] meets these criteria. The CTV encompasses the margins of the resection cavity, including all areas of contrast enhancement plus 5 mm.

DOSAGE AND FRACTIONATION

The optimal dose and fractionation schedule in these patients is unknown. Re-RT is a well-known factor contributing to the risk of RN, which is directly associated with dose and irradiated volume.

Sminia and Mayer[83] examined > 25 glioma re-RT studies to assess tolerance dose and treatment volume of normal brain tissue. RN occurred with a cumulative EQD2 dose ($\alpha/\beta = 3$) > 100 Gy for CFRT, > 105 Gy for fractionated stereotactic radiotherapy (FSRT), and 135 Gy for SRS.

Given that these patients have already received 60 Gy after initial diagnosis, there is a margin of at least 40 Gy for re-RT. Hence, the prescribed doses for re-RT in most published studies ranged from 30-45 Gy, thus maintaining a cumulative EQD2 of approximately 100 Gy[64]. However, given that brain tissue recovers over time, it seems safe to administer higher doses to smaller volumes, using FSRT or SRS, without increasing the likelihood of RN[83].

Scoccianti *et al*[42], after an extensive review of published series and always proposing schemes with reported severe toxicity ≤ 3.5%, described a treatment strategy depending on the volume to be irradiated. Thus, for small volumes (≤ 12.5 mL) SRS schemes are safe (*e.g.*, 12-15 Gy) provided that the EQD2 value does not exceed 65 Gy; HFSRT (*e.g.*, 5 × 5 Gy) for medium-sized lesions (> 12.5-35 mL), provided that the EQD2 value does not exceed 50 Gy and CFRT (*e.g.*, 36 Gy in 20 fr) for larger lesions (> 35-50 mL). These authors pointed out that this recommended strategy should be confirmed in prospective studies.

Whenever possible, hypofractionated schemes are preferred, avoiding unnecessary transfers in these patients with limited life expectancy.

Organ-at-risk tolerance dose

In primary treatment, the maximum doses to the brainstem, chiasm, and optic nerves to avoid the risk of myelopathy are well defined[84,85]. In the context of re-RT in HGG, current evidence is limited[15,44]. Preclinical data suggest a 61% recovery in the spinal cord after 1 year since the first irradiation, and it is believed that this is likely to be applicable to other central nervous system tissues[86]. These models indicate that, in the context of re-RT, maximum summed doses of up to 86 Gy could be tolerated for the optic chiasm and brainstem.

Two series with low recorded toxicity analyzed cumulative dose in organ-at-risk with different doses and fractionations. Shen *et al*[71] reported a median maximum dose in the brainstem of 76.9 Gy and 56 Gy in the optic pathway, with a CFRT schedule and a mean dose of 41.4 Gy. In the series of Chan *et al*[44], with a dose mostly of 35-40 Gy/15 fr, the median maximum dose was 64 Gy for the brainstem and 54.9 Gy for the optic chiasm, although it is noted that concomitant BEV was administered, which may reduce the risk of RN.

It is essential to record and communicate doses to organ-at-risk before re-RTs in order to be able to design a toxicity risk model.

TOXICITY AND QOL

Toxicity

Data on re-RT toxicity are scarce in the literature (Tables 1-4), and its analysis and quantification are difficult. Late toxicity assessment is limited by poor prognosis, difficult differentiation between tumor recurrence, and RN, which is associated with the variety of techniques and fractionations used.

The only existing meta-analysis[33] reported a grade ≥ 3 toxicity rate of 7%, and the morbidity and mortality rate for re-RT ranged from 0%-31% and 0%-1%, respectively.

QoL

Disease progression is associated with deterioration of neurocognitive function. The evidence supporting treatment in this population is evolving, but little is known about its impact on QoL. The survival benefit is desirable but must be carefully weighed against expected morbidities.

Analysis of pooled data from over 300 GBM patients from 13 published articles showed that overall, re-RT resulted in clinical improvement in 24%-45% of patients and a reduction in corticosteroid dependence in 20%-60% of patients. However, the subgroup with KPS < 70 appeared to have a higher risk of early progression and apparently had less benefit from re-RT[87].

Very few studies prospectively evaluate the impact on QoL and activities of daily living in the setting of salvage re-RT. Wick *et al*[88] analyzed QoL in 84 patients with rGBM from a phase II trial with Asunercept/APG 101 and re-RT *vs* re-RT alone, with a dose of 36 Gy at 2 Gy/fr. The EORTC QLQ-C15-PAL, EORTC QLQ-BN20, and Medical Research Council scale questionnaires were used, concluding that Asunercept plus re-RT significantly prolonged time to deterioration of QoL *vs* re-RT alone. More recently, Maitre *et al*[89] reported prospective data on QoL and activities of daily living in patients with recurrent/progressive glioma treated with re-RT (median dose EQD2 51.4 Gy). They used the QLQ-C30 and QLQ-BN20 questionnaires and the modified

Barthel index. They performed 225 evaluations in 60 patients, concluding that high-dose re-RT in selected patients is associated with stabilization of QoL and greater functional independence.

NEW STRATEGIES FOR RE-RT

New re-RT strategies for the treatment of HGG recurrences include particle radiotherapy, as well as intraoperative radiation therapy (IORT) and brachytherapy. Although they are not novel techniques, they are re-emerging in recent years with technological advances.

Particle irradiation

Proton therapy is emerging for the treatment of these patients. Due to its physical and radiobiological properties, this radiation modality offers dosimetric advantages over photons, achieving a better dose distribution and decreasing the irradiation of healthy tissue. The Proton Collaborative Group has published the largest series to date[90]. They analyzed 45 patients with a median of 20.2 mo between initial diagnosis and recurrence. The median dose was 46.2 Gy (range, 25-60 Gy), with a mean of 2.2 Gy/fr, achieving mPFS and mOS of 13.9 and 14.2 mo, respectively. The treatment was well tolerated, and the appreciated toxicity was related to a dose higher than 41 Gy (EQD2). Only prior surgery was positively associated with PFS and OS.

The first study of re-RT with carbon ion beams in rHGG analyzed 30 patients with a median interval between initial radiotherapy and re-RT of 10 mo[91]. The dose administered was 45 Gy in 15 fractions, with a mOS of 13 mo. Eight patients had grade 3 toxicity. Only initial histology with a Ki67 < 20% was a prognostic factor. Resection or chemotherapy did not significantly improve OS. A phase I/II trial to compare re-RT of recurrent gliomas with carbon ions *vs* re-RT with photons is ongoing (NCT 01166308).

IORT

IORT data come from older series, mainly from HGG at diagnosis, and only a few papers included rGBM[32]. The results were promising, but the complexity of the procedure led to abandoning its use. The development of portable systems capable of being moved to the operating room has sparked interest in this technique. This approach is conceptually attractive because it allows the delivery of a large dose of radiation to the tumor bed and tumor debris close to the surgical cavity immediately after resection, while respecting the surrounding brain tissue, decreasing the likelihood of RN. In addition, local and systemic immune responses may be promoted, which could benefit oncological outcomes[92,93].

Recently, although in newly diagnosed GBM, Giordano *et al*[94] reported the results of a phase I/II dose-escalation trial, evaluating the safety and efficacy of the Zeiss INTRABEAM system, a miniaturized 50 keV LINAC with spherical applicators. Fifteen patients, mainly with subtotal resection, were included, receiving a dose of 30 and 40 Gy, with no evidence of limiting toxicity, achieving a PFS of 17.7 mo.

Brachytherapy

Like IORT, it has the advantage of allowing immediate irradiation of the surgical cavity[95], without having to wait the usual 4 wk until the surgical wound is completely healed to start external radiotherapy. This delay is not desirable in HGGs, where in as little as 3 wk there is already a high rate of tumor repopulation. The most commonly used technique is permanent seed implantation. Initially the isotope used was I-125, but high complication rates were reported[96]. Suture-stranded Cs-131 seeds, with a shorter half-life, are now the most commonly used isotope. A study combining re-S with insertion of suture-stranded Cs-131 seeds and BEV (before or after the procedure) has recently been published[95]. Twenty patients were analyzed, with a dose of 80 Gy administered at 0.5 cm from the surface of the resection cavity. Seven patients had been previously salvaged with external radiotherapy. Local control was 85% and mOS was 9 mo. There were two wound infections and three seizures, with no case of RN.

These radiation techniques are safe and effective, but further prospective and comparative research is needed to draw solid conclusions.

SPECIAL PATIENT GROUPS

Elderly patients

As in younger patients, radiotherapy is the cornerstone of first-line treatment of older patients with GBM. However, they receive poor care after recurrence[97]. The evidence for re-RT in older patients is very scarce, as the median age in published papers is around 53 years[33]. However, the aging population is growing and treatment decisions in patients with rGBM and good general condition are increasing. To our knowledge, only one study on re-RT in older patients has been published. Straube *et al* [98] reported the results of 25 patients with a median age of 69.6 years (range 65-79) who received re-RT, most after reintervention. The mOS was 6.9 mo and mPFS at 4.3 mo, with no case of severe toxicity attributable to re-RT. This survival is within the range of series reported in younger patients[28]. Therefore, although prospective trials are needed, these results suggest that second-line salvage therapy should not be dismissed on the basis of age alone.

Pediatric patients

As with adults, children with rHGG have limited treatment options. Re-RT has an emerging role as a palliative treatment for children with recurrent brainstem glioma (diffuse intrinsic pontine glioma or DIPG)[99-101], being associated with symptomatic improvement and longer survival compared to non-re-irradiated patients[99]. Indeed, re-RT in DIPGs is the subject of several ongoing or completed prospective studies (NCT01777633 and NCT03126266). Given that the irradiation dose tolerance of the supratentorial brain is higher than that of the brainstem, it stands to reason that re-RT in supratentorial rHGG should be equally safe and effective[102]. However, the role of re-RT has been little studied in non-pontine gliomas.

Recently, Tsang *et al*[103] have published the results of the largest known cohort of children with recurrent supratentorial HGG treated with re-RT compared to a group of non-re-irradiated children. They retrospectively analyzed 40 patients ≤ 18 years. Fourteen patients, with an interval of at least 6 mo after the first radiotherapy, were re-irradiated. Doses administered ranged from 30-54 Gy at 1.8 Gy/fr. Median survival was 9.4 mo for re-RT patients compared to 3.8 mo for the 26 who did not receive re-RT. The time elapsed between the first and second irradiation determined significant differences, being higher in children with an interval ≥ 12 mo. One patient presented grade 3 RN 4 mo after re-RT. There were no significant differences between patients with initial *vs* distant field re-RT, between those who received concurrent chemotherapy *vs* exclusive re-RT, or between those who were previously operated *vs* those who received radiotherapy alone. Thus, offering re-RT to these patients is associated with reasonable short-term control and survival without significant toxicity.

CONCLUSION

The rHGG scenario remains devastating. Nevertheless, the available evidence, albeit low level, suggests that re-RT, at recommended doses and in selected patients, is safe and provides encouraging local control and survival rates.

The combination of re-S with early re-RT appears to be the most promising option.

Randomized clinical trials are needed to establish the optimal treatment strategy for these patients.

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MINIREVIEWS

Real-world evidence on first- and second-line palliative chemotherapy in advanced pancreatic cancer

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Abstract

In spite of recent diagnostic and therapeutic advances, the prognosis of pancreatic ductal adenocarcinoma (PDAC) remains very poor. As most patients are not amenable to curative intent treatments, optimized palliative management is highly needed. One key question is to what extent promising results produced by randomized controlled trials (RCTs) correspond to clinically meaningful outcomes in patients treated outside the strict frames of a clinical trial. To answer such questions, real-world evidence is necessary. The present paper reviews and discusses the current literature on first- and second-line palliative chemotherapy in PDAC. Notably, a growing number of studies report that the outcomes of the two predominant first-line multidrug regimens, *i.e.* gemcitabine plus nab-paclitaxel (GnP) and folfirinox (FFX), is similar in RCTs and real-life populations. Outcomes of second-line therapy following failure of first-line regimens are still dismal, and considerable uncertainty of the optimal management remains. Additional RCTs and real-world evidence studies focusing on the optimal treatment sequence, such as FFX followed by GnP or vice versa, are urgently needed. Finally, the review highlights the need for prognostic and predictive biomarkers to inform clinical decision making and enable personalized management in advanced PDAC.

Key Words: Pancreatic cancer; Palliative therapy; Cancer chemotherapy; Gemcitabine; Paclitaxel, nano albumin-bound; Folfirinox

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Core Tip: This review summarizes and interprets published real-world evidence of the effectiveness and safety of treatment strategies in advanced pancreatic cancer. The real-world outcomes of first-line chemotherapy regimens such as folfirinox and gemcitabine/ nab-paclitaxel are thoroughly reviewed. The results of randomized controlled trials (RCTs) exploring the regimens seem to be largely generalizable in a real-world context. On second-line options, *i.e.* salvage chemotherapy following failure of first-line therapy, significant uncertainties remain. Additional RCTs and real-world evidence studies addressing current and novel regimens, and the optimal sequence of these, are needed.

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INTRODUCTION

Over the past decades, mortality has decreased for many types of cancer. One exception is pancreatic cancer (pancreatic ductal adenocarcinoma, PDAC), which is soon expected to overtake breast cancer as the second most common cause of cancer-related death[1]. The majority of PDAC patients still present with either locally advanced or metastasized disease, and hence are considered beyond curative potential. For those individuals, as well as for those resected patients who suffer from relapses, palliative systemic therapy and/or radiotherapy are the only treatment options available.

Historically, palliative treatment of PDAC was limited mostly to regimens based on 5-fluorouracil (5-FU), usually with modest results at best. In that setting, 5-FU treatment was more or less experimental, but evidence from a randomized trial in 1996 showed that palliative chemotherapy in PDAC improved median overall survival (mOS) as well as quality of life compared with the best supportive care only[2]. The year after, gemcitabine replaced 5-FU as the gold standard in this clinical scenario based on the results of another randomized trial with prolonged mOS in favor of gemcitabine[3].

For a period of almost 15 years thereafter, many attempts to further improve the treatment in the setting of palliative PDAC were made by adding various cytotoxic drugs and monoclonal antibodies to gemcitabine, often resulting in increased toxicity without any significant survival benefit for patients[4-10]. A first breakthrough came in 2011, when a randomized controlled trial (RCT) showed a significant and clinically meaningful survival benefit over gemcitabine with the triplet combination chemotherapy known as Folfirinox (FFX, 11.1 mo *vs* 6.8 mo for gemcitabine monotherapy)[11]. The survival advantage occurred at the expense of considerably increased hematological and non-hematological toxicity in the intervention group. Another transformative RCT introduced the combination of gemcitabine and nano-albumin-bound paclitaxel (GnP). The regimen produced a smaller effect on overall survival (8.5 mo *vs* 6.8 mo for gemcitabine monotherapy). Nonetheless, it also resulted in increased toxicity, especially myelosuppression and chemotherapy-induced peripheral neuropathy[12]. The finding of more than tripled objective response ratio for the intervention group compared with gemcitabine (roughly 30% *vs* 10%) in both trials indicated a substantial antitumoral effect with the use of those combination regimens. Conversely, the treatment response duration of first-line therapy was usually short, and the RCT-population typically included highly selected patients with lower comorbidity and frailty compared with real-life patients. Whether survival and toxicity data from trials are generalizable to patients treated in routine clinical practice is unclear.

Regarding second-line treatment in PDAC, evidence is scarce. Empirical chemotherapy has been used in highly selected patients, and is usually reserved for very fit or young patients who responded to first-line treatment. Most often, gemcitabine and 5-FU have been used either as monotherapy or in combination with either oxaliplatin or irinotecan. In one of the few RCTs conducted, Oettle *et al*[13]

compared the combination of folinic acid and fluorouracil (FF) in a 42-day cycle with FF in combination with oxaliplatin (OFF). The latter regimen gave significantly longer median progression-free survival (mPFS) and mOS, even though the absolute increase in months was rather small (mPFS 2.0 mo *vs* 2.9 mo, and mOS 3.3 mo *vs* 5.9 mo for FF and OFF, respectively). The occurrence of low-grade neuropathy was more than five times higher (38% of patients) in the OFF group. In contrast, the PANCREOX RCT, which compared the commonly used regimens of 5-FU/leucovorin infusion (5-FU/LV) and modified FOLFOX6 (mFOLFOX6) did not show any advantage with the addition of oxaliplatin[14]. There was no significant difference in the primary mPFS endpoint (3.1 mo *vs* 2.9 mo for mFOLFOX6 and FU/LV). The mOS favored 5-FU/LV (9.9 mo *vs* 6.1 mo for mFOLFOX6). Furthermore, substantial toxicity was observed in the mFOLFOX6-arm, with grade 3-4 adverse events affecting a majority (63%) of participants[14]. A more recent RCT[15] explored the role of 5-FU/LV and liposomal irinotecan in the second-line setting. The combination showed a small survival benefit over 5-FU/LV alone (6.1 *vs* 4.2 mo). However, the 5-FU/LV and liposomal irinotecan combination has not gained widespread traction in countries such as Canada and Sweden because regulatory authorities and health technology assessment bodies have considered the treatment to be not economically justifiable[16-18].

As the results of RCTs may be difficult to interpret and properly implement as standard healthcare, it is essential to complement the basis of knowledge with real-world evidence. The aim of this review was to summarize and assess available studies reporting real-world evidence in support of first- and second-line palliative chemotherapy in advanced PDAC. In first-line therapy, the focus was restricted to the two most established multidrug regimens, *i.e.* FFX and GnP. For second-line therapy, where the evidence on the optimal regimen is weak, no restriction in terms of regimen was applied.

LITERATURE SEARCH

PubMed was searched on December 19, 2020 for studies with titles containing the phrases "pancreatic cancer" and "real world". All results were assessed for potential relevance. Only studies of human pancreatic cancer in the palliative setting and written in English were selected for possible inclusion in this review. Additional requirements for inclusion were information related to chemotherapy (FFX and/or GnP in the first-line setting, or any regimen in the second-line setting); survival [(overall survival (OS) data were required, progression-free survival (PFS) data were optional, and surrogate markers for OS were not accepted); real-world study population, and study type (retrospective or prospective cohort trials). RCTs, published study protocols, case studies, and meeting abstracts were not included. Studies reporting data on several treatment regimens were included as long as either FFX or GnP was among them, and specific survival data and treatment intention for the regimens were clearly distinguishable and compatible with the criteria mentioned above. Included studies are presented in a structured way with key data in tables sorted by topic and year of publication.

RESULTS

The PubMed query on first-line therapy returned 87 publications. Following careful review with regard to the inclusion criteria and scope of this review, 14 articles were selected, four with data on GnP (Table 1), one reporting FFX data (Table 2), eight that compared FFX and GnP (Table 3), and one covering several first-line treatments. The PubMed search of second-line setting returned 17 articles of which 15 were potentially relevant. The articles were subclassified according to which first-line treatment (FFX or GnP) had been administered (Tables 4 and 5). In addition to the above mentioned articles, several papers that did not focus on a specific first- or second-line regimen, and/or described the treatment pattern in general terms, were identified and will be discussed in the relevant section of this review.

First-line GnP combination chemotherapy

Studies evaluating the effect of GnP in the real-world setting are listed in chronological order in Table 1. One study prospectively evaluated the efficacy of the regimen in younger (< 70 years) *vs* older (> 70 years) patients and found no significant between-

Table 1 Real-world studies of gemcitabine/nab-paclitaxel in the first-line setting[19-23]

Ref.	Location	Study design	Stage M1	*n*	Regimen	mOS in mo	Subgroup analysis	mPFS in mo	Remarks
Prager *et al*[19], 2021	Austria	Prospective cohort	100%	317	GnP	10.6/10.2	Age < 70/> 70	5.6/5.5	No difference in frequent toxicities
Blomstrand *et al*[20, 21], 2019/2020	Sweden	Retrospective cohort	71%	75	GnP	10.9	Alb <3 7, age < 65 with shorter survival	5.2	Less hematotoxicity than MPACT
Ostwal *et al*[22], 2018	India	Retrospective cohort	83%	78	GnP	11.6		5.6	Grade III-IV toxicity 35%
Quinton *et al*[23], 2018	United Kingdom	Retrospective cohort	100%	74		8.4		-	Hematotoxicity similar to MPACT

GnP: Gemcitabine/Nab-paclitaxel; M1: Metastatic disease; mOS: Median overall survival; mPFS: Median progression-free survival.

Table 2 Real-world studies of Folfirinox in the first-line setting[24]

Ref.	Location	Study design	*n*	M1	Regimen	mOS in mo	mPFS in mo	Remarks
Cavanna *et al*[24], 2019	Italy	Retrospective cohort	50	74%	FFX/mFFX	10.1	5.6	mFFX sign less toxicity

FFX: Folfirinox; M1: metastatic disease; mFFX: Modified Folfirinox; mOS: Median overall survival; mPFS: Median progression-free survival.

group differences of either mOS (10.6 mo and 10.2 mo) or adverse events[19]. Real-world survival outcomes were superior to those observed in the phase III MPACT trial [12]. The authors suggested that the difference could be explained by a larger fraction of patients proceeding to second-line treatment, 47.4%-56.2% in the real-world studies compared with 38% in the MPACT study[12]. It is also noteworthy that the proportion of patients with performance status (PS) 0 or 1 or corresponding Karnofsky score was somewhat higher than in the MPACT trial. Another study retrospectively evaluated the benefit of GnP in advanced PDAC and found an mOS of 10.9 mo in the entire cohort, and an mOS of 17.1 mo in the locally advanced group[20]. Hematological toxicity was less frequent than in the MPACT study. In the same cohort, multivariate analysis found that low albumin (< 36 g/L) and age (< 65) were significant predictors of worse survival[21].

An additional study found comparable survival outcomes with the use of non-cremophore-based paclitaxel and gemcitabine, with an mOS of 11.6 mo and an mPFS of 5.6 mo[22]. In this retrospective cohort, the majority of patients had metastatic disease (83%) and PS 1 (80%). Grade III-IV toxicity was reported in 36% of patients, with hematological toxicity as the most frequent type of adverse event. In another retrospective cohort analysis where all patients had metastatic disease, mOS was 8.4 mo[23]. Most patients were in PS 1 (66%) at the time of treatment initiation. Similar frequencies of hematological toxicity were seen, with grade III-IV neutropenia being the most frequently reported adverse event (35% of patients).

First-line FFX combination chemotherapy

Studies evaluating the effectiveness of FFX in the real-world setting are listed in chronological order in Table 2. One study evaluated FFX treatment in a retrospective cohort and reported an mPFS of 5.6 mo and an mOS of 10.1 mo[24]. The first 18 consecutive patients received full-dose FFX and the following 32 cases received dose-reduced modified FFX (mFFX), resulting in significantly lower toxicity with fewer hematological and non-hematological side-effects.

First-line FFX vs GnP

Studies comparing the real-world effectiveness of FFX and GnP are listed in chronological order in Table 3. One retrospective cohort study of first-line treatment of patients with metastatic PDAC reported an mOS of 12.7 mo with FFX and 10.2 mo with GnP[25]. Tumor marker serum CA-19-9 and neutrophil-lymphocyte-ratio (NLR) were associated with survival. Authors intended to analyze patients aged above 70 years separately but this group was too small. Hematological toxicity was evenly distributed between the two treatments. Of interest, neuropathy was only reported in

Table 3 Real-world studies comparing Folfirinox and Gemcitabine/Nab-paclitaxel in the first-line setting[25-32]

Ref.	Location	Study design	*n*	M1	Regimen	mOS in mo, *P* value	Prognostic factors	mPFS in mo, *P* value	Remarks
Franco *et al* [25], 2020	Spain	Retrospective cohort	119	50%	FFX 59; GnP 60	FFX 12.7; GnP 10.2; *P* = 0.912	Ca19-9, NLR	-	Toxicity data not reported
Wang *et al* [26], 2019	Canada	Retrospective cohort	225	58%	FFX 92; GnP 87; Gem 46	FFX 14.1; GnP 10.5; Gem 4.2	-	FFX 8.4; GnP 8.5; Gem 3.7	Sign more hematotoxicity in FFX
Pusceddu *et al*[27], 2019	-	Review	3813	NA	FFX 1690; GnP 2123	1.15 longer for FFX. *P* = 0.03	-	-	GnP more neurotoxicity and anemia. FFX more neutropenia
Chiorean *et al*[28], 2019	-	Review	> 6915	NA	FFX > 3556; GnP > 3359	FFX 15.9; GnP 14.4	-	FFX 11.7; GnP 8.5	FFX more neutropenia, GnP more neuropathy
Papneja *et al* [29], 2019	Canada	Retrospective cohort	119	77%	FFX 86; GnP 33	FFX 9.0; GnP 9.0	S-Alb, male sex, 2nd line therapy	FFX 6.0; GnP 4.0	Grade 1-2 thromboembolism, mucositis and neuropathy sign more in FFX. Among grade 3-4 toxicity only fatigue sign more in GnP group
Kordes *et al* [32], 2019	Sweden	Retrospective cohort	595	-	FFX 31; GnP 66; Gem 185	FFX 9.9; GnP 9.8; Gem 6.6	-	-	No sign differences in toxicity comparing FFX *vs* GnP
Cartwright *et al*[30], 2018	United States	Retrospective cohort	486	100%	FFX 159; GnP 255; Gem 72	FFX 11.4; GnP 9.8; Gem 4.4	-	-	No sign differences in toxicity comparing FFX *vs* GnP
Kim *et al* [31], 2018	United States	Retrospective cohort	654	100%	FFX 317; GnP 337	FFX 13.8; GnP 12.1; *P* = 0.96	Age	-	Less toxicity in GnP group

FFX: Folfirinox; Gem: Gemcitabine; GnP: Gemcitabine/Nab-paclitaxel; M1: Metastatic disease; mOS: Median overall survival; mPFS: Median progression-free survival; NA: Not applicable; NLR: Neutrophil-leucocyte ratio; s-Alb: Serum albumin.

two patients receiving FFX. A study that compared the real-world effectiveness of FFX, GnP and gemcitabine reported OS durations of 14.1, 10.5 and 4.2 mo for the three treatments, respectively[26]. FFX treated patients were significantly younger and had better PS, and OS was significantly longer in both FFX- and GnP-treated patients compared with gemcitabine. The majority of patients had metastatic disease (68%). For the subgroups with localized disease, median OS had not been reached at the time of publication. The occurrence of neutropenia, febrile neutropenia, and neuropathy was significantly more frequent in FFX treated patients. In a review article, slightly longer survival (an additional 1.2 mo) was noted in favor of FFX over GnP. Despite the numerical difference, the overall adjusted risk of death was similar regardless of the regimen administered[27]. Neurotoxicity and anemia were seen more frequently in GnP-treated patients; neutropenia was more often associated with FFX treatment. In another review, a similar, non-significant, survival benefit was seen for FFX, with a reported OS of 15.9 mo *vs* 14.4 mo for GnP[28]. PFS was 11.7 mo with FFX and 8.5 mo for GnP. Toxicity data were not consistently reported in the studies, but neutropenia was more often associated with FFX than with GnP. The opposite was observed for neuropathy. In a retrospective study that largely focused on metastatic PDAC patients (77%), equivalent survival for FFX and GnP was reported (OS 9.0 mo for both regimens, P = 0.88). However, PFS was slightly longer with FFX, although the difference was not statistically significant (6.0 mo for FFX *vs* 4.0 mo for GnP, P = 0.38) [29]. There were no significant differences in the frequencies of severe toxicity between the two regimens. Another retrospective study reported OS of 11.4, 9.8 and 4.4 mo for FFX, GnP and gemcitabine monotherapy, respectively. Again, the differences were not significant[30]. Patients receiving GnP were significantly older and had worse PS. Toxicities were evenly distributed between the treatment groups. No significant prognostic factors were found in multivariate analysis, except for PS 2+, which was associated with worse survival. In another Celgene-funded real-world retrospective cohort study, there was a slight, non-significant, trend that favored FFX over GnP, with an OS of 13.8 mo compared with 12.1 mo[31]. All patients had metastatic disease. Common side-effects such as nausea, vomiting, diarrhea and mucositis were less

Table 4 Real-world studies of second-line therapy following failure of Folfirinox[36-44]

Ref.	*n*	M1	2L regimen	mPFS in mo	mOS in mo	Remarks	AE
Portal *et al*[36], 2015	57	100%	GnP	5.1	8.8	Prospective cohort	38% grade 3-4 toxicity
Mita *et al*[37], 2019	30	80%	GnP	3.8	7.6	Phase II	70% grade 3-4 toxicity
Tsang *et al*[38], 2019	159	67%	GnP 78; Gem 81	-	5.8; 4.6	Population-based, three Canadian provinces	-
Zhang *et al*[39], 2018	60	73%; 75%; 73%	GnP 30; Gem 8; BSC 22	3.6; 2.5	5.7; 3.8	Single center	More grade 3-4 fatigue in Gem
Nguyen *et al* [40], 2017	30	77%	GnP	3.7	12.4	Single center	Grade 3-4 thrombocytopenia (33%), anemia (23%), nausea (17%)
Bertocchi *et al* [41], 2015	23	100%	GnP	3.0	5.0	Single center	-
Zhang *et al*[42], 2015	28	82%	GnP	3.0	5.7	Single center	Grade 3-4, anemia (25%), thrombocytopenia (25%), neutropenia (18%)
Caparello *et al* [44], 2016	71	-	GnP	2.5	6.2	Single center	-
Rissy *et al*[43], 2017	12	100%	GnP	4.9	-	Single-center	No grade 3-4 toxicity reported

BSC: Best supportive care; Gem: Gemcitabine; GnP: Gemcitabine/Nab-paclitaxel; M1: metastatic disease; mOS: Median overall survival; mPFS: Median progression-free survival.

Table 5 Real-world studies of second-line treatment with Folfirinox following failure of gemcitabine/nab-paclitaxel or single-agent gemcitabine[46-51]

Ref.	*n*	M1	1L regimen	2L regimen	mPFS in mo	mOS in mo	Remarks
Sawada *et al*[46], 2020	104	100%	GnP	Modified; FFX	3.9	7.0	Bolus 5-FU omitted. 55% grade 3-4 toxicity
Matsumoto *et al* [47], 2020	23	83%	GnP	FFX 12; mFFX 11	5.3; 4.3	6.9; 12.8	No sign difference in toxicity between FFX/mFFX
Assaf *et al*[50], 2011	27	100%	Gem	FFX	3.0	8.5	56% grade 3-4 neutropenia
Kobayashi *et al* [48], 2017	18	100%	Gem	FFX	2.8	9.8	Phase I/II. 83% grade 3-4 toxicity
Kim *et al*[51], 2018	39	82%	Gem	Attenuated; FFX	3.8	8.5	Oxaliplatin: 65 mg/m^2. 41% grade 3-4 neutropenia
Chung *et al*[49], 2018	48	79%	Gem	Reduced irinotecan and oxaliplatin; FFX	5.8	9.0	Phase IIIrinotecan: 120 mg/m^2; Oxaliplatin: 60 mg/m^2; 65% grade 3-4 neutropenia

5-FU: Fluorouracil infusion; FFX: Folfirinox; Gem: Gemcitabine; GnP: Gemcitabine/Nab-paclitaxel; M1: Metastatic disease; mFFX: Modified Folfirinox; mOS: Median overall survival; mPFS: Median progression-free survival.

frequent in the GnP group. A Swedish retrospective study comparing palliative first-line treatment in a PDAC patient cohort that included 31 FFX, 66 GnP, and 185 gemcitabine patients reported OS of 9.9, 9.8 and 6.6 mo, respectively[32]. Patient characteristics, including age and PS, varied substantially among the three groups. No significant differences in grade 3 or higher toxicities were reported between FFX and GnP.

Second-line real-world studies

Second-line treatment in PDAC: Despite advancements in the first-line treatment of advanced PDAC, most patients progress and succumb to the disease. To date, three phase III randomized clinical trials have been reported in the second-line treatment space[13-15] and are thoroughly described above under the background heading.

These three trials compared 5-FU alone *vs* 5-FU/oxaliplatin doublets[13,14] or 5-FU *vs* nal-irinothecan *vs* 5-FU/nal-irinothecan doublet[15], and were all conducted after the patients progressed on gemcitabine-based chemotherapy as first-line treatment for advanced PDAC. However, the contemporary first-line standard treatment includes FFX or GnP combinations for patients with good PS[11,12]. There are no randomized clinical trial data for second-line treatment specifically after failure on FFX and GnP. Second-line treatment of advanced pancreatic cancer is largely driven by the chemotherapy regimen administered in the first-line setting. In a large real-world study that examined the outcome of 167 patients with advanced PDAC using several treatment regimens, the mOS from start of second-line therapy (OS2) was 5.2 mo, and plasma albumin, serum CA-19-9, and performance status were identified as key prognostic factors[33].

Second-line treatment after first-line FFX: In the real world, such patients are usually treated with GnP combination or gemcitabine monotherapy. The initial supportive evidence for use of GnP after first-line use of FFX in advanced pancreatic cancer was published in the form of case reports[34,35]. Subsequently, a prospective multicenter cohort study of 57 patients treated with GnP after FFX failure reported an mPFS of 5.1 mo and an OS2 of 8.8 mo[36]. It is noteworthy that just over half of the patients who received FFX for advanced pancreatic cancer in the frontline setting were eligible to receive salvage therapy with GnP in this cohort study. The objective response rate was 17.5%, while the disease control rate was 58.0%. From the start of first-line chemotherapy, the median OS was 18.0 mo. Grade 3-4 toxicities were observed in 40.0% of patients, of which neutropenia and neuropathy were the two most common. Recently, a phase II study of 30 patients reported in this setting described an mPFS of 3.8 mo and an OS2 of 7.6 mo[37]. The corresponding figures from the start of first-line chemotherapy were 9.3 and 14.2 mo, respectively. The overall response rate was 13.3% and the disease control rate was 46.7%. Grade 3-4 toxicities were reported in 70.0% patients, the most common being neutropenia and neuropathy. Furthermore, several real-world studies have been reported to support the use of GnP as second-line treatment. A large population-based Canadian study compared the real-world data of 368 patients with advanced PDAC treated with first-line FFX across two provinces with differential access to second-line treatment[38]. Of these, 159 patients (43.2%) received second-line treatment that was equally allocated as GnP (49.1%) and single-agent gemcitabine (50.9%). In a secondary analysis, the mOS counted from the initiation of second-line chemotherapy (OS2) was slightly longer for GnP compared with (5.8 mo *vs* 4.6 mo, $P = 0.01$).

Another Canadian study included 60 patients with advanced PDAC who received FFX as the first-line treatment[39]. Of these, 30 patients (50.0%) were treated with GnP, 8 (13.3%) with gemcitabine alone, and 22 patients (37.7%) received optimal supportive care. The mPFS (3.6 mo *vs* 2.5 mo, $P = 0.03$), and OS2 (5.7 mo *vs* 3.8 mo, $P = 0.03$) were longer in patients who received GnP compared with gemcitabine (Table 4). Other real-world studies have reported similar PFS and OS2 with the use of GnP after failure of FFX[40-44]. Furthermore, a recently published systematic review that included 16 studies reported a higher overall response rate (14.4% *vs* 8.4%, $P = 0.038$), disease control rate (53.5% *vs* 30.2%, $P < 0.001$), PFS (3.6 mo *vs* 2.5 mo, $P = 0.030$), and OS2 (5.7 mo *vs* 3.8 mo, $P = 0.030$) with GnP than with gemcitabine monotherapy[45]. Similar grade 3/4 event rates were reported in the prespecified analysis (22.9% *vs* 34.6%, $P = 0.415$). Overall, GnP appears to be a reasonable second-line treatment after FFX and patients considered unfit for GnP may benefit from gemcitabine monotherapy, while those with a poor performance status should be offered the best supportive care.

Second-line treatment after first-line GnP: In the absence of a head-to-head comparison of FFX and GnP in advanced PDAC, a substantial proportion of patients are treated with GnP in the first-line setting. Several chemotherapy regimens using a combination of fluoropyrimidines with irinotecan and/or oxaliplatin have been used in the real-world as salvage, second-line therapy of such patients. It is intuitive to consider FFX in this setting. A recent retrospective analysis of 104 patients treated with modified FFX (*i.e.* intravenous oxaliplatin 85 mg/m^2, intravenous irinotecan 150 mg/m^2, and continuous infusion of 5-fluorouracil 2400 mg/m^2 for 46 h without bolus infusion) in that setting reported an objective response rate of 10.6% and a disease control rate of 56.7%[46]. The median PFS and OS2 were 3.9 mo and 7.0 mo, respectively. Grade 3-4 adverse events were reported in 54.8% patients and included hematological toxicities and peripheral sensory neuropathy. A smaller study of 23 patients who received standard FFX ($n = 12$) and modified FFX ($n = 11$) reported a median PFS of 5.3 mo and an OS of 6.9 mo in patients who received standard dosages.

The corresponding numbers for those receiving modified FFX were 4.3 and 12.8 mo, respectively[47]. The observed differences in survival between the FFX and mFFX groups were not statistically significant.

Other real-world studies have reported the effectiveness of either the standard or modified FFX regimen after failure of single-agent gemcitabine as first-line therapy[48-51]. The studies, which adopted several modifications of the original FFX regimen, reported a PFS of 2.8-5.8 mo and OS2 of 8.5-9.8 mo (Table 2). Overall, limited data from real-world studies supports the use of modified FFX after failure of GnP. However, it is an intensive chemotherapy regimen and a high rate of grade 3-4 adverse events has been reported in above-mentioned studies, primarily hematological events and peripheral neuropathy. Patient selection remains paramount for electing to use such a regimen.

A real-world study of 52 patients with gemcitabine-refractory advanced PDAC reported that nano-liposomal irinotecan with FF was associated with a median PFS of 3.8 mo and OS2 of 6.8 mo[52]. The figures closely mirror the outcome reported from the phase III NAPOLI-1 study[15]. Capecitabine combined with oxaliplatin has also been used in this setting,, and several studies have reported a PFS of around 3 mo and OS2 of approximately 6 mo[53-55]. The median PFS and OS with single-agent capecitabine in 41 patients who failed first-line therapy were reported to be 1.5 mo and 4.3 mo, respectively[56].

Therefore, in patients considered unfit for FFX as second-line treatment, a doublet chemotherapy with fluoropyrimidine and oxaliplatin or nano-liposomal irinotecan is reasonable, while monotherapy with capecitabine may be considered for those with borderline performance status. There are no clinical trials that have compared the efficacy of oxaliplatin with irinotecan in this setting. However, a meta-analysis reported that the combination of a fluoropyrimidine plus irinotecan significantly improved both PFS and OS2, while the oxaliplatin combination modestly improved PFS but not OS2[57]. The modest benefit with these regimens should be balanced with the associated adverse events, and best supportive care should be considered a viable option for patients with poor general condition.

Targeted therapy and immunotherapy

As survival is still short, even when the most effective modern combinations of cytotoxic drugs are administered to patients with good performance status, it is tempting to look for alternatives such as targeted therapies or immune checkpoint inhibitors for the treatment of advanced PDAC. While the major breakthrough is yet to come, some recent findings may have the potential to become game-changing treatments of at least some types of PDAC in the future.

Approximately one in every five patients with advanced PDAC harbors a germline or somatic mutation in the DNA damage repair pathway[58]. There are limited data to suggest that Poly (ADP-ribose) polymerase (PARP) inhibitors may be effective in such patients. For example, a retrospective analysis of patients with previously treated PDAC (median prior therapies = 2) harboring a mutation in the DNA damage repair pathway reported an objective response rate of 23%, PFS of 7.6 mo and OS of 16.5 mo with olaparib[59]. Another report of 30 patients with BRCA1/2 mutations and no available standard treatment options reported disease control rate of 31% and an objective response rate of 4% with olaparib[60]. The role of immunotherapy in advanced PDAC is still evolving. However, a low prevalence (< 2%) of deficient mismatch repair suggests a limited role of immune check point inhibitors in this setting, at least with the currently available drugs[61,62].

CONCLUSION

Pancreatic cancer not amenable to surgical resection remains one of the most difficult challenges for medical oncologists around the globe. Despite improved diagnostic imaging tools, most cases are detected at a stage where cure or long-term survival are not achievable. Nevertheless, there is reason for cautious optimism. Large RCTs over the last decade have introduced first-line FFX and GnP regimens as the current standard of care, which has significantly changed the treatment landscape. Although extrapolation of the outcomes observed in highly selected RCT populations should be done with great care, combined evidence from real-world studies across different countries and health care systems indicates that the regimens are effective and reasonably safe in the real-world setting. In several of the real-world evidence public-

ations, FFX was associated with a slightly better median OS than GnP, but selection bias was probable. Thus, it is possible that the differences observed might be the result of less fit patients being prescribed GnP rather than FFX. A sufficiently large head-to-head RCT comparing first-line FFX and GnP would potentially resolve these issues, but such a study is unlikely to occur.

In terms of second-line therapies, there are still considerable gaps in our knowledge. The few available RCTs provide only limited guidance, and it is difficult to translate their results into real-life practice. Notably, none of the published RCTs addresses whether the sequence of FFX followed by GnP or GnP followed by FFX is the most feasible or beneficial approach. Still, those sequences are often advocated by expert guidelines, and several real-world experience studies support that strategy. The extrapolation of RCTs into the real world is, at least in theory, even more complex in the second-line setting because patients at that point in their disease trajectory are likely to be frailer than patients eligible for first-line therapy.

The accumulating real-world evidence presented in this review points to some key conclusions. Several multidrug regimens show promising potency and acceptable toxicity in the first-line scenario, and to a somewhat lesser extent, the second-line setting. Outcomes reported in RCTs seem to be relatively consistent when the respective regimens are administered in real-life patients. Larger and/or pooled real-world studies are needed to further explore prognostic and predictive parameters such as serum albumin, serum CA-19-9, NLR and other novel biomarkers. Regarding second-line chemotherapy, the RCTs and real-world studies published to date are not fully aligned, and the key question regarding the optimal sequence of regimens remains uncertain. While most patients in this situation have very short expected survival, the identification of reliable clinical and biochemical biomarkers could be very helpful to inform treatment decision making.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Long-term results of the treatment of Hodgkin's lymphoma in a resource-constrained setting: Real-world data from a single center

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Abstract

BACKGROUND

The outcomes of Hodgkin´s lymphoma (HL) in Mexico have not been widely reported. Simplified and affordable treatments have been adopted in middle-income countries.

AIM

The aim was to evaluate long-used therapies for HL in Mexico in a long-term basis.

METHODS

In a 34-year time period, 88 patients with HL were treated at a single institution in Mexico. Patients were treated with adriamycin bleomycin vinblastine and dacarbazine (ABVD) or mechlorethamine, vincristine, procarbazine, and prednisone (MOPP). Relapsed or refractory patients were given ifosfamide, carboplatin, and etoposide (ICE) followed by autologous or allogeneic stem cell transplants.

RESULTS

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Thirty-seven women and 51 men were included; the median age was 29 years. Patients were followed for a mean of 128 mo. The 310-mo overall survival (OS) was 83% for patients treated with MOPP and 88% for those treated with ABVD. The OS of patients who received autologous stem cell transplantation was 76% (330 mo) *vs* 93% (402 mo) in those who did not.

CONCLUSION
HL may be less aggressive in Mexican population than in Caucasians. Combined chemotherapy renders acceptable results, regardless of clinical stage.

Key Words: Hodgkin; Lymphoma; Treatment; ABVD chemotherapy

Core Tip: In a retrospective, observational long-term study, our group found that the treatment of Hodgkin lymphoma in a resource-constrained background may still rely on the use of the traditional adriamycin bleomycin vinblastine and dacarbazine treatment regimen in order to achieve acceptable outcomes. The observations were consistent across different stages of disease and may serve to propose new studies focusing on the comparison of newly approved therapies in contexts where there are some healthcare limitations.

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INTRODUCTION

Hodgkin´s lymphoma (HL) is the model of curative care, with radiation therapy, combination chemotherapy, staging approaches, peripheral blood-stem cell transplantation, and immunotherapy[1]. Following the initial demonstration that radiotherapy could eradicate limited-stage disease, multiagent chemotherapy regimens proved to be curative in a large proportion of patients with advanced disease [1]. In the 40 years since De Vita and colleagues developed the mechlorethamine, vincristine, procarbazine, prednisone (MOPP) chemotherapy regimen, much has been learned about risk stratification to minimize treatment-related toxicity[2]. Doxorubicin (*i.e.* adriamycin), bleomycin, vinblastine, and dacarbazine (ABVD), the most commonly used regimen for both early and advanced stage HL, was developed in the mid-70s[2] and continues to be a standard of care in HL[3]. In recent years, there have been advances, with the introduction of novel therapies and changes in the management algorithms[1]. However, the performance of newer therapies remains unclear in real-world conditions, especially for overall survival (OS) and quality of life of persons with malignant diseases[4]. We analyze here the results of the treatment of a group of 88 patients with HD over a 34-year period at a single institution, treated with combined chemotherapy in a resource-constrained setting of a single institution.

MATERIALS AND METHODS

Patients

All consecutive patients seeking medical care for HL at our institution after 1986 and followed for at least 3 mo were entered into the study. A diagnosis of HL was based on the histological study of a pathology specimen, mainly a lymph node; the same pathologist analyzed all the specimens and defined the histological subtype[5]. The

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clinical stage was defined according to the Ann Arbor classification[5]. Bone marrow biopsies were done only in patients with clinical stages III or IV[6]. Computed tomography (CT) scans were done in all cases prior to starting treatment. Fluorodeoxyglucose positron emission tomography (FDG-PET) scans have been performed since 2002. The study was approved by the institutional review board, and all participants signed an informed consent.

Treatment

Between 1986 and 1997, patients were treated with MOPP, *i.e.* nitrogen mustard (6 mg/m^2 on days 1 and 8 of the cycle), vincristine (1.4 mg/m^2 on days 1 and 8), procarbazine (100 mg/m^2 on days 1 through 14) and prednisone (40 mg/m^2 during cycles 1 and 4 for 14 d)[7]. After 1997, patients were treated with ABVD, *i.e.* doxorubicin (25 mg/m^2), vinblastine (6 mg/m^2), dacarbazine (375 mg/m^2) and bleomycin (10000 units/m^2) on days 1 and 15 of every 4 wk[8] as frontline therapy. Per local protocol, stages I and II, were treated with four cycles of chemotherapy and the response was assessed by a CT scan. If disease activity persisted at that time, four additional cycles were given, whereas two additional cycles were given if the CT scan was negative. For stages III and IV, the CT scans were performed after six cycles, and two or four more cycles were delivered depending on the results, as described above. Bleomycin was administered only in the first three courses and the doxorubicin dose was adjusted to avoid delivering more than 450 mg/m^2. FDG-PET scans have been performed at the end of treatment since 2002. Scans were not performed between cycles. Only patients with disease activity at the end of treatment received radiotherapy. Patients showing activity after treatment were considered as refractory and treated with four courses of ifosfamide, carboplatin and etoposide (ICE)[9]. Autologous or allogeneic peripheral blood hematopoietic stem cell transplant (HSCT) were given to refractory patients after achieving complete remission. High-dose melphalan (200 mg/m^2) was used in autologous transplants; cyclophosphamide, fludarabine, and busulfan were used in allogeneic transplants, which were all from HLA-identical siblings[10]. After the completion of treatment, patients were follow-up every 2 mo for 1 year and every 4 mo from then on. No FDG-PET scans were done during follow-up, unless clinically indicated.

Statistical analysis

The primary outcome measure was OS, defined as the time elapsed between the diagnosis of HL and death from any cause, with censoring of patients who were alive on the last follow-up date. Differences were assessed with Fisher's exact test. OS was estimated by the Kaplan-Meier method, and differences between groups were compared with the log-rank test[11]. Two-sided P values < 0.05 were considered statistically significant. The statistical analysis was carried preformed with Prism 8 (GraphPad Inc. San Diego, CA, United States).

RESULTS

Patient characteristics

Of the 91 patients with HL identified between 1986 and 2020, 88 were followed for 3 mo or more and were included in the analysis. There were 37 women and 51 men. The median age was 29 years (range: 5-73 years). There were 62 patients with nodular sclerosing HL (70%), 19 with mixed cellularity HL, two with lymphocyte depleted HL, and one with lymphocyte predominant HL. In four cases, the histologic variant could not be defined. According to the Ann Arbor classification[5], five patients were stage I, 48 were stage II, 19 were stage III, and 16 were stage IV. Ten patients presented with a mediastinal mass larger than 10 cm in the chest X-ray film. Three cases presented with relapsed disease (Table 1).

Treatment patterns

As frontline therapy, all patients were offered chemotherapy (ChT). Twelve received MOPP and 70 received ABVD; three were treated with initial radiotherapy (RT): two refused ChT, and one was referred after receiving RT. Relapsed or refractory patients were treated with ICE and a subsequent autologous or allogeneic HSCT.

Responses

Patients were followed for a median of 114 mo (range: 4-402). Forty-four are alive, ten

Table 1 Salient features of 88 patients with Hodgkin´s lymphoma

Sex	Women	37 (42)
	Men	51 (57.9)
Age, yr	Median 29 (range: 5-73)	
Type	Nodular sclerosing	62 (70)
	Mixed cellularity	19 (21.5)
	Lymphocyte depleted	2 (2.2)
	Lymphocyte predominant	1 (1.1)
Stage	Stage I	5 (5.6)
	Stage II	48 (54.5)
	Stage III	19 (1.5)
	Stage IV	16 (18.1)

Data are *n* (%)

have died, and 34 were lost to follow-up. Median OS for all patients has not been reached, and is more than 402 mo. OS was 88% 310 mo and 77% 402 mo (Figure 1). Median OS has not been reached and is 94 mo for stage I, 109 mo for stage II, 90 mo for stage III, and 98 for stage IV (P = 0.2). The 310-mo OS was 83% for patients treated with MOPP and 88% for those treated with ABVD [hazard ratio (HR): 0.76, 95% confidence interval (CI): 0.2-2.8, P = 0.6; Figure 2]. Sixteen patients (18%) were refractory to treatment and nine (10%) relapsed. They were treated with ICE followed by HSCT, autologous in 15 patients and allogeneic in ten patients. Patients who underwent autologous HSCT had a median survival of 329 mo and an OS of 92%. Those given allogeneic HSCT had a median survival of 59 mo and an OS of 46% (HR: 0.2, 95%CI: 0.04–1.3, P = 0.057). The OS of patients given and HSCT was 73% at 266 mo and was 93% at 404 mo in those not given HSCT (HR: 4.09, 95%CI: 1.0–16.6, P = 0.01) (Figure 2B). The OS was similar (Figure 2). The causes of death were breast carcinoma in two cases, liver carcinoma in one, and uncontrolled lymphoma activity in the remaining patients.

Long-term toxicity

Twelve patients developed peripheral neuropathy. There were no reported cases of pulmonary, fertility, or cardiovascular toxicity. Five patients developed a secondary neoplasia 18-150 mo after completing treatment; four had received chemotherapy (three ABVD and one MOPP); one had received radiotherapy alone. The salient features of the patients are shown in Table 2.

DISCUSSION

The outcomes of HL in Mexico have not been fully analyzed or reported. We have previously shown that some malignancies in Mexico have different behaviors in the population of Mexican mestizos compared with other populations. For example, multiple myeloma in Mexico is less frequent[12,13] and less aggressive than in Caucasians or African-Americans[12], and chronic lymphocytic leukemia is also less frequent and less aggressive in Mexican mestizos than in other populations[14-16], but promyelocytic leukemia is substantially more frequent in Mexico than in Caucasian populations[14,17]. In the case of HL in Mexico, there is not enough information about its prevalence and clinical behavior. Preliminary data indicate that the prevalence of HL in Mexico is similar to that reported in other populations[17,18]. The data presented here suggest that the clinical picture of the disease may be less aggressive in Mexico, as without employing novel and sophisticated drugs, the OS of this group of patients was 88% at 310 mo, including all stages of the disease, relapsed, and refractory patients (Figure 1). There were no significant differences between patients treated with MOPP or ABVD. The OS of relapsed or refractory patients who were given an HSCT was significantly lower than those who did not require it (Figure 3).

Table 2 Salient features of patients who developed a secondary malignancy after the treatment of lymphoma

	Neoplasm	Age	Sex	Type	Stage	Treatment	HSCT	Time[1], mo
1	Tongue epidermoid carcinoma	32	M	Nodular sclerosing	I	Radiotherapy	No	58
2	Liver adenocarcinoma	22	F	Nodularsclerosing	III	MOPP	No	97
3	Breast cancer	38	F	Mixed cellularity	III	ABVD	No	150
4	NK/T-cell lymphoma	15	M	Nodular sclerosing	IV	ABVD	Autologous	18
5	Breast cancer	23	F	Nodular sclerosing	III	AVBD	Autologous	59

[1]Time in months elapsed between the diagnosis of Hodgkin's lymphoma and the diagnosis of the secondary neoplasia.
HSCT: Hematopoietic stem cell transplant; M: Male; F: Female.

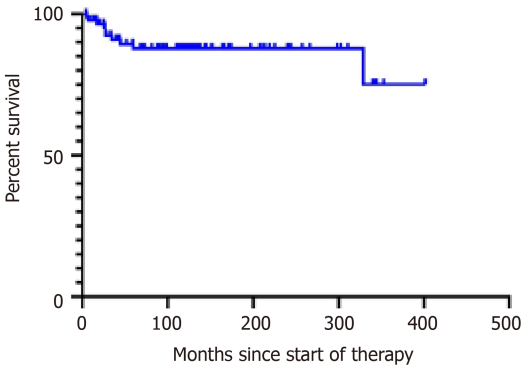

Figure 1 Overall survival of 88 patients with Hodgkin's lymphoma.

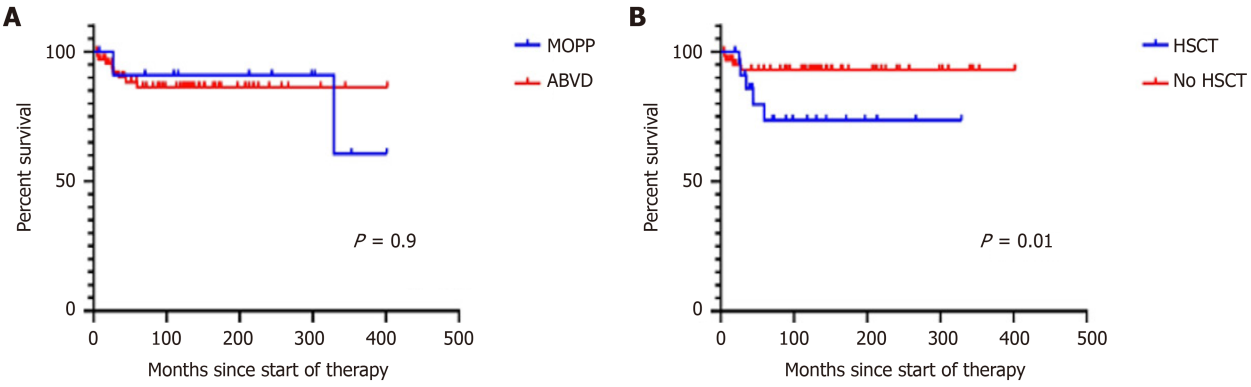

Figure 2 Overall survival of 88 patients with Hodgkin's lymphoma treated with either MOPP or ABVD (A) and treated either with or without hematopoietic stem cell transplants (B). ABVD: adriamycin bleomycin vinblastine and dacarbazine; HSCT: Hematopoietic stem cell transplant; MOPP: mechlorethamine, Oncovin, procarbazine, and prednisone.

Radiation therapy is included in treatment strategies. We have previously suggested that in Mexico, and probably in other underprivileged circumstances where RT facilities are suboptimal, patients with early stages of HL should be treated with ChT alone. The message being "conventional ChT is better than a poor RT"[19,21-24]. The results that we present here support the previous observations. Additionally, patients with relapsed/refractory disease were successfully rescued with ICE followed by HSCT. Figure 3 shows the OS of the patients classified by the prognostic score described by Hayden *et al*[24].

The main observations of treatment which we present here are (1) ABVD was offered to all patients regardless of their clinical stage. (2) Four cycles were administered to patients with stages I and II and six cycles to those with stages III and IV. (3) Two additional cycles were given to all patients after a negative CT scan after receiving 4-6 cycles. (4) No interim FDG-PET scans were done, reserving them for the

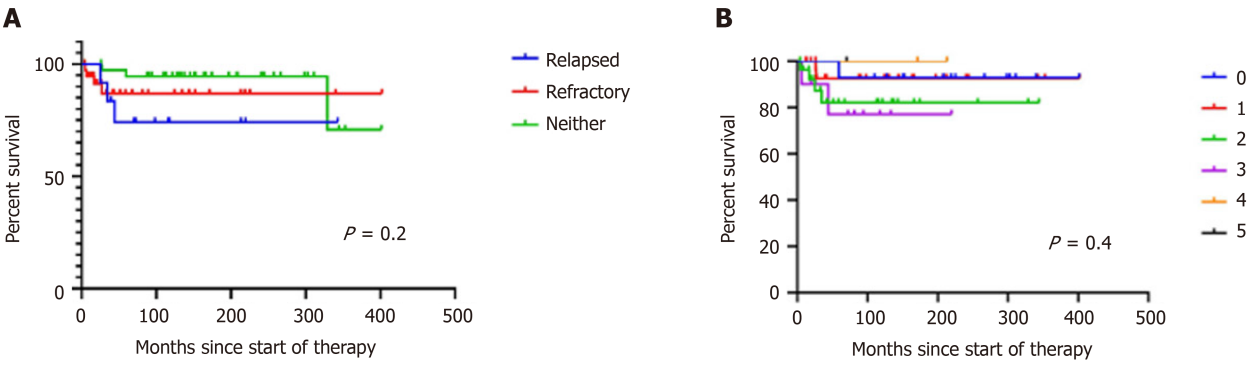

Figure 3 Overall survival of 88 patients with Hodgkin's lymphoma with or without relapsed or refractory disease (A), and overall survival of the patients with Hodgkin's lymphoma classified as described by Diefenbach *et al* (B).

end of treatment. And (5) Relapsed or refractory patients were given ICE followed by an HSCT. The limitations of this study include a relatively small and heterogeneous sample, the potential of referral bias, a high proportion of patients lost to follow-up, and socioeconomic factors.

This simplified approach to the treatment of patients with HL demonstrates adequate results with an OS of 88% at 26 years regardless of the clinical stage or relapsed/refractory disease. Additional data are needed to confirm the observations, which may be useful in circumstances of a restrained economy. The use of novel and expensive drugs such as pembrolizumab, nivolumab, panabinostat, idelalisib, mocetinostat, brentuximab vedotin and others[4,24] should be reserved for multi-relapsed cases and does not appear to be essential as frontline therapy.

CONCLUSION

HL may be less aggressive in the Mexican than in Caucasian populations. Combined chemotherapy without radiotherapy achieves acceptable results. In our context with healthcare limited-resources, chemotherapy alone with ABVD continues to be the treatment of choice in patients with HL.

ARTICLE HIGHLIGHTS

Research background

Hodgkin´s lymphoma (HL) can be treated with different alternatives, the performance of newer and older chemotherapy schemes are unknown in some circumstances.

Research motivation

The motivation was to describe the performance of treatment of HL in a middle-income country.

Research objectives

The objective was to determine performance of classic therapies for HL.

Research methods

This was a comparative study of therapies for HL in a single center over a long-term period.

Research results

HL may be less aggressive in the Mexican population. In addition, the classical ABVD regimen achieved long-term survival in a significant proportion of patients.

Research conclusions

Combined chemotherapy has acceptable efficacy in patients with HL. Our results suggest that classical treatment schemes continue to be an effective alternative. More

studies should be conducted.

Research perspectives

Classic therapies for HL may still be preferable over novel therapies in middle-income countries. The use of ABVD combined with other immunomodulatory agents could be a potential solution for patients not experiencing favorable outcomes.

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ORIGINAL ARTICLE

Retrospective Study

Role of mammogram and ultrasound imaging in predicting breast cancer subtypes in screening and symptomatic patients

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Abstract

BACKGROUND

Breast cancer (BC) radiogenomics, or correlation analysis of imaging features and BC molecular subtypes, can complement genetic analysis with less resource-intensive diagnostic methods to provide an early and accurate triage of BC. This is pertinent because BC is the most prevalent cancer amongst adult women, resulting in rising demands on public health resources.

AIM

To find combinations of mammogram and ultrasound imaging features that predict BC molecular subtypes in a sample of screening and symptomatic patients.

METHODS

This retrospective study evaluated 328 consecutive patients in 2017-2018 with histologically confirmed BC, of which 237 (72%) presented with symptoms and 91 (28%) were detected *via* a screening program. All the patients underwent mammography and ultrasound imaging prior to biopsy. The images were retrospectively read by two breast-imaging radiologists with 5-10 years of experience with no knowledge of the histology results to ensure statistical independence. To test the hypothesis that imaging features are correlated with tumor subtypes, univariate binomial and multinomial logistic regression models were performed. Our study also used the multivariate logistic regression (with and without interaction terms) to identify combinations of mammogram and ultrasound (US) imaging characteristics predictive of molecular subtypes.

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RESULTS

The presence of circumscribed margins, posterior enhancement, and large size is correlated with triple-negative BC (TNBC), while high-risk microcalcifications and microlobulated margins is predictive of HER2-enriched cancers. Ductal carcinoma *in situ* is characterized by small size on ultrasound, absence of posterior acoustic features, and architectural distortion on mammogram, while luminal subtypes tend to be small, with spiculated margins and posterior acoustic shadowing (Luminal A type). These results are broadly consistent with findings from prior studies. In addition, we also find that US size signals a higher odds ratio for TNBC if presented during screening. As TNBC tends to display sonographic features such as circumscribed margins and posterior enhancement, resulting in visual similarity with benign common lesions, at the screening stage, size may be a useful factor in deciding whether to recommend a biopsy.

CONCLUSION

Several imaging features were shown to be independent variables predicting molecular subtypes of BC. Knowledge of such correlations could help clinicians stratify BC patients, possibly enabling earlier treatment or aiding in therapeutic decisions in countries where receptor testing is not readily available.

Key Words: Hormone receptor; Molecular subtype; Ultrasonography; Mammography; Triple-negative cancer; Breast cancer screening

Core Tip: Ultrasound and mammogram imaging features are correlated with breast cancer (BC) molecular subtypes. Knowledge of such correlations helps clinicians stratify patients, enabling earlier treatment or aiding therapeutic decisions. In a sample of symptomatic and asymptomatic (screening) patients, multivariate logistic regression showed that a combination of imaging features can distinguish: (1) Hormone receptor positive *vs* hormone receptor negative; (2) Triple negative BC (TNBC) *vs* non-TNBC; and (3) HER2+ (human epidermal receptor positive) *vs* non-HER2+ BC.

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INTRODUCTION

Breast cancer (BC) radiogenomics has been a prolific field of research in recent years, with an expanding number of studies examining the extent to which imaging can be utilized as a screening adjunct in the preliminary diagnosis of BC molecular subtypes [1]. While magnetic resonance imaging (MRI) has been the mainstay of radiogenomics, as it can produce a wide range of imaging features, a few studies have also utilized readily available modalities, such as mammogram (MG) and hand-guided ultrasound (US). These highlight the advantages of using cost-effective imaging tools that can be used for an early and accurate diagnosis of the cancer subtype[2].

The use of non-invasive, less resource-intensive methods to predict BC subtypes has practical significance, for two reasons. First, as BC is the most common cancer amongst adult women in the world, leveraging on radiologic imaging as a proxy for expensive genetic tests may help to lower the public health burden, particularly in the context of less developed countries where detailed and costly histopathologic analysis is not readily available. Second, BC is a heterogeneous disease—receptor expression and gene amplification profiles have different prognoses for disease progression and therapeutic response. Thus, radiologic imaging as an adjunct to genetic profiling can assist in pre-treatment planning and provide an additional level of analysis for radio-pathologic correlation discussions.

Prior research has established some degree of consensus regarding the correlation of imaging features with BC subtype, with ultrasound margins, posterior acoustic features, and high-risk microcalcifications (on MG) found to be independent imaging differentiators between molecular subtypes[3].

While previous studies focused mainly on symptomatic patients, this study aims to analyze which combination of US and MG imaging features are the most predictive of molecular subtypes in a patient population that comprises both symptomatic and asymptomatic (breast screening) patients. A unique feature of our dataset is a substantial proportion—slightly under 30% of the patients in our sample patients had cancers detected *via* our national screening program (91 of 328, 28%). This allows us to examine if imaging features indicative of higher-grade cancers can be detected in asymptomatic patients who undergo routine screening checks. In addition, due to the inclusion of screening patients, a significant proportion of cancers detected in our sample included non-invasive ductal carcinoma *in situ* (DCIS) (or 53 of 328, 16%). Thus, we were also able to analyze its mammographic and sonographic characteristics in relation to the four subtypes of invasive BCs, which to the best of our knowledge, has not been published in the existing literature.

MATERIALS AND METHODS

Patient data and selection criteria

Approval for this study was obtained from the local institutional review board. The need for informed consent for this study was waived due to the retrospective design, as patients had given permission to be included in the hospital's BC registry and the use of their unidentified data for research.

To minimize selection bias, our sample included consecutive patients with histologically confirmed BC diagnosed from January 2017 to December 2018. The surgical notes, histology reports, and medical images of 328 patients were retrieved *via* the hospital's electronic medical record system. The images were retrospectively read by two breast-imaging radiologists with 5-10 years of experience with no knowledge of the histology results to ensure statistical independence with regards to the dependent variable(s).

Imaging equipment and assessment

Standard two-view digital mammography was performed, with additional views when necessary, using Fuji or GE mammography units. Mammograms were interpreted by two breast imaging radiologists who were blinded to the histopathology report. Following the American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS) reporting lexicon, abnormal mammographic findings were classified as an asymmetry, mass, architectural distortion, or suspicious microcalcifications.

Breast ultrasound was performed by ultrasound technologists using 12-5 Mhz transducers, with images interpreted by two breast imaging radiologists who were blinded to the histopathology report. Final decisions were made with consensus agreement among the radiologists. Each tumor was measured in three planes, with color Doppler images also acquired. Following the ACR BI-RADS guidelines, the lesion's margins, echogenicity, posterior features, and vascularity (Adler's index) were documented, along with size on ultrasound (Table 1).

Immunochemistry and classification of molecular subtypes

The expression status of the ER, PR, and HER2 receptors was assessed using an avidin-biotin complex immunohistochemical technique. The ER/PR statuses were evaluated using the Allred score based on the proportion of positively stained nuclei. Allred scores of at least 3 were considered as hormone receptor positive (ER+/PR+). The HER2 staining intensity was scored from 0 to 3+. Scores of 3+ were classified as HER2 positive (HER2+), whereas scores of 0/1+ were considered as HER2 negative (HER2-). Tumors with scores of 2+ were further assessed with fluorescence *in situ* hybridization to determine their HER2 status. The threshold ratio of the HER2 gene signal to the chromosome 17 probe signal was 2.2, above which the tumor was classified as HER2+ and below which the tumor was classified as HER2-.

For this paper, we have classified Luminal A tumors as being ER+/PR+ and HER2-, Luminal B tumors as being ER+/PR+ and HER2+, HER2-enriched as ER-/PR- and HER2+, and triple negative BC (TNBC) as being ER-/PR- and HER2-.

Table 1 Classification of imaging features based on American College of Radiology Breast Imaging Reporting and Data System Lexicon

Imaging feature	Classification
Mammogram	
Appearance	Mass
	Asymmetry
	High risk microcalcifications[1]
	Architectural distortion
Ultrasound	
Margins	Spiculated
	Microlobulated
	Circumscribed
Posterior acoustic features	Shadowing
	Enhancement
	Mixed
	None
Size	Maximum dimension on ultrasound (in mm)
Echogenicity	Homogenous
	Heterogeneous
	Complex cystic
Adler's index	Low (Grade I)
	Medium (Grade II)
	High (Grade III)

[1]High risk microcalcifications refer to microcalcifications which show grouped, linear, or segmental distribution, or with a pleomorphic or branching morphology.

Statistical analysis

Descriptive statistics were generated for the 328 patients included in the study. Clinicopathologic, mammogram, and ultrasound characteristics were based on the ACR BI-RADS lexicon. Data was summarized using frequency and percentage for qualitative variables, mean ± SD, and range for quantitative variables, as listed in Table 2.

To test the primary hypothesis that imaging features are correlated with tumor subtypes, univariate binomial and multinomial logistic regression models were performed. Our study also used the multivariate logistic regression (with and without interaction terms), to assess if a joint combination of MG and US imaging characteristics is predictive of molecular subtypes, and the results are summarized in Tables 3-6. The specification for the multivariate model was derived using stepwise regression (SLE = 0.05, SLS = 0.05). Discrimination and classification of the final multivariate models or predictor were assessed using the area under the receiver operating characteristic curve (AUC). All analysis was performed with the use of statistical software (RStudio, Version 1.3.1073), using the mlogit (v.1.1-0; Croissant, 2002), nnet (v2.13.1; Venables & Ripley, 2002)[4,5], and pROC (Robin *et al*[6]) packages.

RESULTS

Out of the 328 cases, 139 (48%) were ER+/PR+, HER2- (Luminal A type); 38 (12%) were ER+/PR+, HER2+ (Luminal B type); 50 (15%) were ER-/PR-, HER2+ (HER2-enriched type); 48 (15%) were ER-/PR-, HER2- (triple negative type), and 53 (16%) were DCIS. The age range of the study sample was 31-86-years-old, with a mean age of 61.1 years. Across the four molecular subtypes, there was no significant difference in age. Ninety-one (27%) cases were detected *via* breast screening, while the remaining

Table 2 Distribution of demographic and imaging parameters based on molecular subtype

	Total (*n* = 328)	DCIS (*n* = 53)	Luminal A (*n* = 139)	Luminal B (*n* = 38)	Her2 enriched (*n* = 50)	Triple negative (*n* = 48)
Mean age	61.1 ± 11.75	59.1 ± 11.67	61.8 ± 11.80	60.2 ± 12.23	62.0 ± 9.63	61.3 ± 12.60
Presentation						
Clinic	237 (72%)	29 (55%)	98 (71%)	28 (74%)	36 (72%)	46 (96%)
Screening	91 (28%)	24 (45%)	41 (29%)	10 (26%)	14 (28%)	2 (4%)
Mass	200 (61%)	14 (26%)	91 (65%)	29 (76%)	29 (58%)	37 (77%)
Architectural distortion	17 (6%)	7 (13%)	10 (7%)	0 (0%)	0 (0%)	0 (0%)
Asymmetry	40 (12%)	6 (11%)	16 (12%)	3 (8%)	9 (18%)	6 (13%)
High-risk microcalcifications	105 (32%)	27 (51%)	33 (24%)	12 (32%)	24 (48%)	9 (19%)
Tumor size (on USG)						
< 20	136 (41%)	22 (42%)	71 (51%)	20 (53%)	19 (38%)	4 (8%)
≥ 20	154 (47%)	9 (17%)	55 (40%)	17 (45%)	29 (58%)	44 (92%)
Margins (on USG)						
Spiculated	100 (30%)	5 (9%)	63 (45%)	12 (32%)	8 (16%)	8 (17%)
Microlobulated	174 (60%)	23 (43%)	59 (42%)	25 (66%)	40 (80%)	29 (60%)
Circumscribed	16 (5%)	3 (6%)	4 (3%)	0 (0%)	0 (0%)	11 (23%)
Echogenicity (on USG)						
Heterogeneous	144 (44%)	13 (25%)	58 (42%)	19 (50%)	28 (56%)	26 (54%)
Homogenous	146 (45%)	18 (34%)	68 (49%)	18 (47%)	20 (40%)	22 (46%)
Posterior acoustic features						
Shadow	74 (23%)	5 (9%)	49 (35%)	10 (26%)	4 (8%)	6 (13%)
Enhancement	85 (26%)	5 (9%)	23 (17%)	8 (21%)	6 (32%)	33 (69%)
Mixed	36 (11%)	5 (9%)	15 (11%)	3 (8%)	9 (18%)	4 (8%)
None	95 (29%)	16 (30%)	39 (28%)	16 (42%)	19 (38%)	5 (10%)
Adler's vascularity						
High (Grade II & III)	81 (25%)	7 (13%)	22 (16%)	11 (29%)	21 (42%)	20 (42%)
Low (Grade I)	209 (64%)	24 (45%)	104 (75%)	26 (68%)	27 (54%)	28 (58%)
Axillary nodes						
Present	56 (17%)	0 (0%)	25 (18%)	1 (3%)	14 (28%)	16 (33%)
Absent	234 (71%)	53 (100%)	101 (73%)	36 (95%)	34 (68%)	32 (67%)
Not visible on US	38 (14%)	22 (42%)	13 (9%)	1 (3%)	2 (1%)	0 (0%)

DCIS: Ductal carcinoma *in situ*; US: Ultrasound; USG: Ultrasonography.

191 (69%) cases presented with symptoms at the breast clinic. Most of the cases (89%) detected *via* breast screening were classified as DCIS or hormone receptor positive cancer, and only a minority (11%) of cases detected *via* screening were hormone receptor negative cancers.

Microcalcifications

High risk microcalcifications were found in slightly under one third of the full sample (n = 105, 32%) but were more prevalent amongst BC patients with the subtype HER2-enriched (n = 24, 48%) and DCIS (n = 27, 51%) (Figure 1A). In the univariate multinomial logistic regression (with reference to Luminal A as the baseline), the presence of high risk microcalcifications on mammography was positively associated

Table 3 Binomial univariate and multivariate logistic regressions (ER/PR positive *vs* negative)

ER/PR positive *vs* negative		Univariate			Multivariate (AUC = 0.792)		
		P	OR	CI	*P*	OR	CI
Posterior acoustic features[1]	Enhancement	[d]	0.46	(0.193 1.050)	[d]	0.45	(0.174 1.143)
	Shadowing	[b]	4.26	(1.617 11.633)	Not significant		
Margins[2]	Spiculated	[a]	4.16	(2.268 7.975)	[a]	0.41	(1.085 4.606)
	Circumscribed	[a]	0.15	(0.023 0.594)	Not significant		
Size	Small < 20 mm	[c]	4.51	(2.499 7.443)	[a]	2.74	(1.475 5.204)

[1]Mixed posterior acoustic features were set as the baseline with OR = 1.
[2]Microlobulated ultrasound margins were set as the baseline with OR = 1.
[a]Significant at the 0.001 level.
[b]Significant at the 0.01 level.
[c]Significant at the 0.05 level.
[d]Significant at the 0.10 level.
Area under the receiver operating characteristic curve for multivariate regression on posterior acoustic features, ultrasound margins, MM high-risk microcalcifications, and size (large or small).

Table 4 Binomial univariate and multivariate logistic regressions (Triple-negative breast cancer *vs* Non-triple-negative breast cancer)

TNBC *vs* Non-TNBC		Univariate			Multivariate (AUC = 0.853)		
		P	OR	CI	*P*	OR	CI
Posterior acoustic features[1]	Enhancement	[b]	5.08	(1.808 18.201)	[a]	4.77	(1.556 18.291)
Margins[2]	Spiculated	[a]	0.43	(0.179 0.951)	Not significant		
	Circumscribed	[c]	11.00	(3.712 37.166)	[b]	8.24	(2.151 38.923)
Size	Large > 20 mm	[c]	13.2	(5.152 44.845)	[c]	10.5	(3.792 38.436)
Axillary node metastasis	Yes	[b]	2.52	(1.247 4.988)	Not significant		
Adler's Index	High	[a]	2.12	(1.104 4.020)	Not significant		
[a]Screening	Yes	[d]	0.003	(0.000 0.222)	[d]	0.004	(0.000 0.452)
[a]Interaction term	Screen × unit size	[d]	1.17	(1.003 1.505)	[d]	1.16	(1.001 1.430)

[1]Mixed posterior acoustic features were set as the baseline with OR = 1.
[2]Microlobulated ultrasound margins were set as the baseline with OR = 1.
[a]Significant at the 0.001 level.
[b]Significant at the 0.01 level.
[c]Significant at the 0.05 level.
[d]Significant at the 0.10 level.
Area under the receiver operating characteristic curve for multivariate regression on posterior acoustic features, ultrasound margins, and size (large or small). AUC: Area under the receiver operating characteristic curve; TNBC: Triple-negative breast cancer.

with the presence of HER2 receptor expression (P = 0.002, OR 2.97) and DCIS ($P \leq$ 0.001, OR 3.34). In the multivariate logistic regression, high-risk microcalcifications also increase the relative odds of the tumor being a HER2-enriched type ($P \leq$ 0.001, OR 3.38) (Table 5).

Architectural distortions

Architectural distortions presented rarely in DCIS (n = 7, 13%) and Luminal A subtypes (n = 10, 7%), and were not common in the other three subtypes of BC. On univariate analysis, architectural distortions were significantly associated with DCIS (P = 0.024, OR 3.23) (Table 6), but no statistically significant relationship with Luminal A type was found.

Margins

The vast majority of tumors in our study (n = 274, 94%) had non-circumscribed

Table 5 Binomial univariate and multivariate logistic regressions (HER2+ *vs* Non HER2+)

HER2+ *vs* Non HER2+		Univariate			Multivariate (AUC = 0.747)		
		P	OR	CI	*P*	OR	CI
Margins[1]	Microlobulated	[c]	3.92	(1.830 9.410)	[b]	3.26	(1.469 8.026)
	Circumscribed	Not significant			Not significant		
High-risk microcalcifications	Present	[c]	3.51	(1.804 6.821)	[c]	3.38	(1.685 6.816)
Adler's Index	High	[a]	2.32	(1.203 4.437)	[a]	1.99	(0.999 4.010)

[1]Spiculated ultrasound margins were set as the baseline with OR = 1.
[a]Significant at the 0.001 level.
[b]Significant at the 0.01 level.
[c]Significant at the 0.05 level.
[d]Significant at the 0.10 level.
Area under the receiver operating characteristic curve for multivariate regression on ultrasound (US) margins, MM high-risk microcalcifications, and US Adler index (low or intermediate/high). AUC: Area under the receiver operating characteristic curve.

Table 6 Binomial univariate and multivariate logistic regressions (Ductal carcinoma *in situ* *vs* Invasive cancers)

DCIS *vs* Invasive cancers		Univariate			Multivariate (AUC = 0.719)		
		P	OR	CI	*P*	OR	CI
Posterior acoustic features[1]	None	[a]	3.24	(1.204 10.292)	[a]	3.45	(1.255 11.118)
High-risk microcalcifications	Present	[b]	2.80	(1.516 5.208)	Not significant		
Architectural distortions	Yes	[b]	5.34	(1.546 16.709)	Not significant		
Size	Small < 20 mm	[a]	2.59	(1.420 7.361)	[a]	2.72	(1.172 6.182)

[1]Posterior acoustic enhancement was set as the baseline with OR = 1.
[a]Significant at the 0.001 level.
[b]Significant at the 0.01 level.
[c]Significant at the 0.05 level.
[d]Significant at the 0.10 level.
Area under the receiver operating characteristic curve for multivariate regression on posterior acoustic features, MM high-risk microcalcifications, architectural distortions, and size (large or small). AUC: Area under the receiver operating characteristic curve; DCIS: Ductal carcinoma *in situ*.

margins, of which more than half were classified as microlobulated (n = 174, 60%), followed by spiculated margins (n = 100, 34%). Only 6% of all lesions showed circumscribed margins, of which the majority were TNBC cases (n = 11, 4%), and the rest were DCIS (n = 3, 1%). Based on the univariate and multivariate logistic regressions, the presence of circumscribed margins was found to significantly increase the relative odds of TNBC cancer (univariate: P = 0.003, OR 11.0; multivariate: P = 0.004, OR 8.24) (Figure 2A, Table 4). Moreover, the presence of a spiculated margin on sonography was common in Luminal A cancers (n = 67, 53%), but rarely presented in TN and HER2+ tumors (n = 16, 16%). Thus, if the tumor shows spiculated margins, the odds of it being a hormone receptor positive subtype is higher, as confirmed in the univariate and multivariate regressions (univariate: P = 0.000, OR 4.16; multivariate: P = 0.03, OR 2.20) (Table 3).

Posterior acoustic features

Posterior acoustic enhancement was more common in HER2-enriched and TN tumors (n = 16, 32% and n = 33, 69%), compared to luminal cancers (n = 31, 19%) and DCIS (n = 5, 9%). It is a strong predictor for TNBC cancers *vs* non-TNBC cancers (univariate: P = 0.005 OR 5.08; multivariate: P = 0.01 OR 4.77) (Figure 2B, Table 4). In the multinomial logistic regressions, posterior enhancement signaled higher relative odds of the tumor being a TNBC cancer relative to Luminal A, DCIS, and HER2-enriched tumors (Table 4).

Conversely, posterior acoustic shadowing is associated with slightly higher odds of the tumor being a hormone positive type. In Luminal A cancers, posterior shadowing (n = 49, 39%) was more prevalent compared to the other three subtypes of invasive

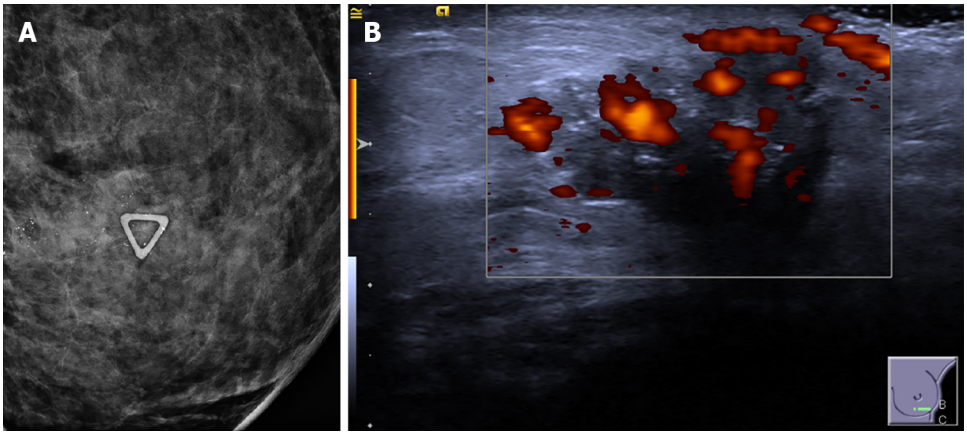

Figure 1 HER2-enriched invasive cancer. A: High-risk microcalcifications on mammogram of the left breast are seen within the palpable mass (denoted by triangular skin marker); B: Irregular hypoechoic mass showing increased internal vascularity (Adler Index Grade III) and internal echogenic foci representing microcalcifications.

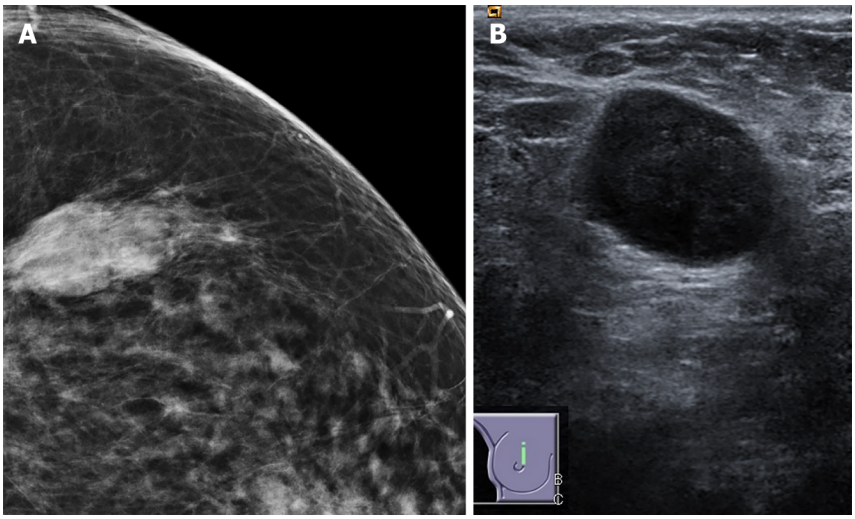

Figure 2 Triple negative breast cancer. A: Circumscribed mass on mammogram; B: Circumscribed hypoechoic mass with posterior acoustic enhancement on ultrasound.

cancer (n = 20, 15%). This was confirmed in the univariate binomial and multinomial regressions, posterior acoustic shadowing suggested a higher likelihood of the tumor being a hormone receptor positive type (univariate: P = 0.037, OR 4.26) (Table 3). However, there was no statistically significant positive association in the multivariate model specifications, possibly due to the lack of statistical power given the limited sample size.

Of the DCIS tumors that were visible on US (60% of all DCIS), the majority did not show any posterior acoustic features (n = 16, 51%). In comparison, most of the invasive cancers showed either posterior shadowing, enhancement, or a mix of both and only a minority showed no posterior acoustic features (n = 79, 29%). Absence of posterior acoustic features favors DCIS in the univariate and multivariate regressions (univariate: P = 0.028, OR 3.24; multivariate: P = 0.090, OR 2.49) (Table 6).

Vascularity

Tumors with a high score on the Adler index (II or III) comprised a minority of the sample (n = 55, 23%), and were more predominant in the HER2 enriched and TNBC subtypes (n = 21, 42%, n = 20, 42%, respectively) (Figure 1B). High vascularity was statistically significant as a predictor of HER2-enriched status in both the multivariate and univariate regressions (univariate regression: P = 0.011, OR 2.32; multivariate regression: P = 0.05, OR 1.99) (Table 5), while low vascularity is more likely in Luminal A tumors, corroborating similar findings in other studies[3] (Table 3). Similar to another study which showed a positive association between TNBC and high

vascularity using optoacoustic US imaging[7], we also found a positive association between TNBC cancers and vascularity in the univariate, but not multivariate specifications (univariate: $P = 0.022$, OR 2.12) (Table 4).

Tumor size

Tumor size was one of the strongest predictors of TNBC. If the lesion is large (> 20 mm), the relative odds of the tumor being a TNBC subtype would be 13 times higher compared to a non-TNBC type (univariate regression: $P \leq 0.001$, OR 13.20; Multivariate regression: $P \leq 0.001$, OR 10.54) (Table 4). In the multinomial regressions, large tumor size consistently predicted a higher likelihood of the tumor being TNBC *vs* all other subtypes. The high positive correlation between size and TNBC status reflects the fact that almost all the TNBC tumors were large ($n = 44$, 92%), compared to around 40% of the Luminal A/B tumors, and 60% of the HER2 enriched tumors. Large tumor size also signaled a higher likelihood of HER2-enriched status *vs* DCIS and Luminal A in the multinomial regression ($P = 0.008$, OR 3.73; $P = 0.05$, OR 1.97) (Table 7).

In contrast, sonographically visible DCIS and hormone receptor positive cancers are typically small (< 20 mm). In the univariate and multivariate regressions, small size increases the relative likelihood of the tumor being a DCIS type by 2.5-3 times, compared to invasive cancers (univariate: $P = 0.006$, OR 2.59; multivariate: $P = 0.012$, OR 3.02) (Table 6). Within the invasive cancer subtypes, a small sized tumor signals a higher likelihood of the tumor being either Luminal A/B (hormone receptor positive), compared to HER2-enriched or TNBC (univariate: $P \leq 0.001$, OR 4.26; multivariate: $P = 0.002$, OR 2.74) (Table 3).

Axillary node metastasis

Axillary node metastasis was most common in TNBC cancers ($n = 16$, 33%), followed by Luminal A ($n = 25$, 18%), and least prevalent in the Luminal B subtype ($n = 1$, 3%). We found evidence that TNBC is associated with a higher positive rate of axillary adenopathy compared to the non-TNBC tumors in the univariate binomial logistic regression ($P = 0.008$, OR 2.53) (TNBC *vs* non TNBC) but not the multivariate binomial logistic regression (Table 4). In the univariate multinomial model, the presence of axillary adenopathy represented a higher likelihood of the tumor being TNBC ($P = 0.029$, OR 18.50) or Luminal A subtype ($P = 0.043$, OR 3.08) relative to Luminal B subtype (Table 4). Our results are in keeping with several studies who also recorded a positive association between the presence of axillary node metastases and TNBC cancers[8,9]. However, the findings on axillary adenopathy and TNBC association have been mixed in the literature, with some other studies finding a negative association instead[10].

Multivariate logistic regressions

Although the univariate regressions show that a few key radiologic imaging features are statistically significant as independent prognostic indicators of the BC subtype, using a joint combination of imaging features could increase the reliability of the preliminary diagnosis. This is because US image acquisition is highly user dependent, and radiologic interpretation may sometimes be equivocal, for instance, in the case of lobulated (microlobulated) *vs* angular (spiculated) margins.

Similar to previous studies[7,11], we estimated multivariate binomial logistic regressions using the stepwise regression approach (SLE = 0.05, SLS = 0.05), with the final model specification determined by the Akaike information criterion. We catalogue four distinct categorizations: (1) Hormone-receptor positive *vs* hormone-receptive negative invasive cancers; (2) TNBC *vs* non-TNBC invasive cancers; (3) HER2-enriched *vs* non HER2-enriched invasive cancers; and (4) DCIS *vs* invasive cancers. The distinguishing characteristics for each category are: (1) A small lesion (< 20 mm), with spiculated margins, the absence of posterior acoustic enhancement and absence of high-risk microcalcifications, is more likely associated with hormone-receptor positive status (Figure 3); (2) Posterior acoustic enhancement, circumscribed margins, and large tumor size was predictive of TNBC status; (3) Microlobulated margins, high-risk microcalcifications, and high vascularity was predictive of HER2-enriched status; and (4) Absence of posterior acoustic features and small size on ultrasound was associated with DCIS compared to invasive cancers. A receiver operating characteristic curve was plotted using the pROC package in R; the performance of the multivariate models based on the AUC was (1) 0.792; (2) 0.853; (3) 0.747; and (4) 0.719, respectively.

Table 7 Multinomial univariate logistic regressions: Impact of imaging features on relative odds of molecular subtypes

			Luminal A baseline			Luminal B baseline			DCIS baseline			HER2 enriched baseline			TNBC baseline		
			P value	OR	95%CI	*P* value	OR	95%CI	*P* value	OR	95%CI	*P* value	OR	95%CI	*P* value	OR	95%CI
Posterior acoustic features	Shadowing	Luminal A *vs*							[d]	**3.27**	**(0.832 12.83)**	[b]	**7.35**	**(1.979 27.300)**			
		Luminal B *vs*										[a]	**7.50**	**(1.307 43.030)**			
		DCIS *vs*	[d]	0.31	(0.078 1.202)												
		HER2+ *vs*	[b]	0.14	(0.037 0.505)	[a]	0.13	(0.023 0.765)									
		TNBC *vs*															
	Enhancement	Luminal A *vs*													[b]	0.19	(0.055 0.633)
		Luminal B *vs*															
		DCIS *vs*													[a]	0.12	(0.024 0.610)
		HER2+ *vs*													[a]	0.22	(0.058 0.807)
		TNBC *vs*	[b]	**5.38**	**(1.581 18.310)**				[a]	**8.25**	**(1.638 41.55)**	[a]	**4.64**	**(1.239 17.38)**			
Margins (on US)	Spiculated	Luminal A *vs*				[a]	**2.45**	**(1.130 5.31)**	[b]	**5.41**	**(1.931 15.14)**	[c]	**5.88**	**(2.544 13.58)**	[c]	**4.26**	**(1.805 10.056)**
		Luminal B *vs*	[a]	0.41	(0.188 0.885)							[d]	**2.40**	**(0.861 6.690)**			
		DCIS *vs*	[b]	0.19	(0.066 0.518)												
		HER2+ *vs*	[c]	0.17	(0.074 0.393)	[d]	0.42	(0.150 1.16)									
		TNBC *vs*	[c]	0.24	(0.099 0.554)												
	Circumscribed	Luminal A *vs*													[b]	0.09	(0.019 0.445)
		Luminal B *vs*															
		DCIS *vs*															
		HER2+ *vs*															
		TNBC *vs*	[b]	**10.8**	**(2.246 52.043)**												
Size	Large	Luminal A *vs*										[a]	0.51	(0.258 0.999)	[c]	0.07	(0.024 0.208)
		Luminal B *vs*													[c]	0.08	(0.023 0.259)
		DCIS *vs*													[c]	0.04	(0.010 0.134)
		HER2+ *vs*	[a]	**1.97**	**(1.001 3.878)**										[c]	0.14	(0.043 0.450)
		TNBC *vs*	[c]	**14.20**	**(4.811 41.915)**	[c]	**12.94**	**(3.856 43.427)**	[c]	**26.89**	**(7.445 97.114)**	**c**	**7.21**	**(2.224 23.354)**			
High-risk	Present	Luminal							[c]	0.30	(0.154	[b]	0.34	(0.171			

Microcals		A *vs*								0.583)			0.665)			
		Luminal B *vs*						[d]	0.44	(0.186 1.061)						
		DCIS *vs*	[c]	**3.34**	**(1.715 6.488)**	[d]	**2.25**	**(0.942 5.37)**						[b]	**4.50**	**(1.606 9.96)**
		HER2+ *vs*	[b]	**2.97**	**(1.504 5.844)**									[b]	**4.00**	**(1.824 11.10)**
		TNBC *vs*						[b]	0.22	(0.090 0.548)		0.25	(0.100 0.623)			
Adler	High	Luminal A *vs*									[c]	0.27	(0.131 0.566)	[b]	0.30	(0.142 0.618)
		Luminal B *vs*														
		DCIS *vs*									[d]	0.38	(0.136 1.037)	[d]	0.41	(0.147 1.131)
		HER2+ *vs*	[c]	**3.68**	**(1.767 7.650)**			[d]	**2.67**	**(0.965 7.37)**						
		TNBC *vs*	[b]	**3.38**	**(1.618 7.045)**			[d]	**2.45**	**(0.884 6.78)**						
Axillary node adenopathy	Yes	Luminal A *vs*				[a]	**3.08**	**(1.063 61.96)**						[a]	0.44	(0.209 0.919)
		Luminal B *vs*	[a]	0.12	(0.224 0.470)						[a]	0.07	(0.009 0.556)	[b]	0.05	(0.007 0.430)
		DCIS *vs*														
		HER2+ *vs*				[a]	**14.40**	**(1.798 115.17)**								
		TNBC *vs*	[a]	**2.28**	**(1.088 4.778)**	[b]	**18.50**	**(2.323 147.34)**								

[a]Significant at the 0.001 level.
[b]Significant at the 0.01 level.
[c]Significant at the 0.05 level.
[d]Significant at the 0.10 level.
Only statistically significant results are shown. Bold results show higher relative odds. DCIS: Ductal carcinoma *in situ*; TNBC: Triple-negative breast cancer; US: Ultrasound.

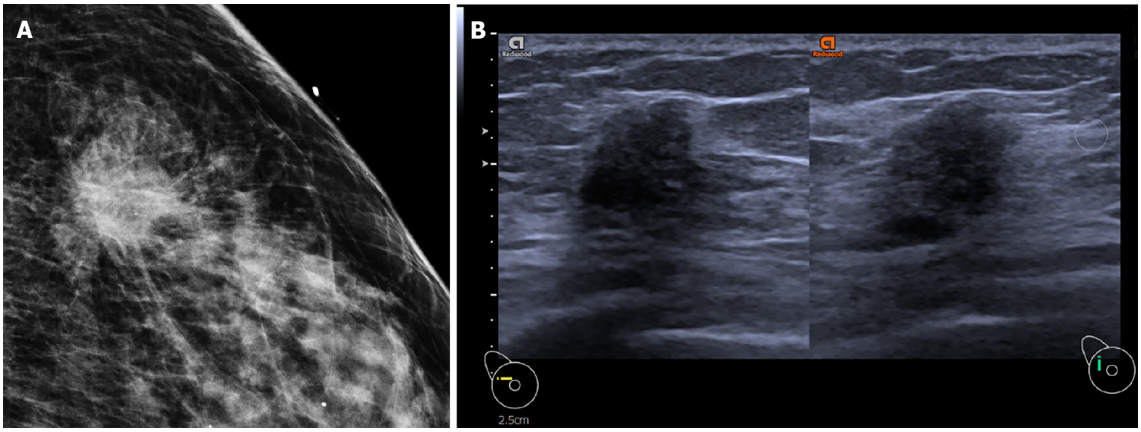

Figure 3 Luminal type invasive cancer. A: Spiculated mass on mammogram; B: Irregular hypoechoic mass with spiculated margin and posterior acoustic shadowing.

Screening and size of tumor

A significant proportion of the cancers in our sample were detected *via* routine breast screening (n = 91, 28%). Previous studies have shown that BCs detected *via* screening are typically smaller than symptomatic BCs, and that screening is an effective means of

detecting tumors at an earlier stage[12].

In our sample, screening detected tumors when they were smaller, even after controlling for molecular subtype. The mean size of hormone receptor negative tumors detected *via* screening was 19.0 mm *vs* 33.0 mm for hormone receptor negative cancers presenting with symptoms. The mean size of TNBC and HER2-enriched cancers that were detected *via* screening was 32.5 mm and 14.4 mm respectively *vs* 34.7 mm and 30.8 mm, respectively for symptomatic patients. However, as size is a less important predictor of disease severity than the biologic characteristics of the tumor based on its gene expression, a few prominent studies have downplayed the usefulness of breast screening in improving patient outcomes, considering its tendency for overdiagnosis of in-situ type cancers[13].

One of the main findings of our study is that TNBC cancers tend to have benign morphologic features, such as circumscribed margins and posterior enhancement. This raises possibility of mistaking it for common benign lesions such as cysts with echoes or fibroadenomas. The only unique differentiator is its positive correlation with size. Thus, in the screening context, size may be a useful factor in deciding whether to recommend an invasive biopsy or imaging follow-up for the lesion in question.

To estimate the impact of size on the probability of the tumor being a TNBC subtype conditional on the tumor presenting during screening, in the multivariate regressions, we specified an interaction term of screening with size, and find a marginally significant positive coefficient for the interaction term. Interpretively, this means that for every unit increase in lesion size, there are higher relative odds of the screening tumor being a TNBC subtype compared to non-TNBC, holding all other factors unchanged (P = 0.08, OR 1.18) (Table 4).

DISCUSSION

BC is a heterogenous disease, characterized by the varied imaging appearance, histologic and molecular profiles, and correspondingly different disease course across the various molecular subtypes. The different molecular types of BC have different biological behaviors at the cellular level, which influence the speed of invasion and destruction of the surrounding tissue, consequently affecting the macroscopic appearance of the tumor on mammogram and ultrasound.

Prior studies investigating the association of imaging features with molecular subtypes found evidence that cancers with posterior acoustic shadowing have higher odds of hormone-receptor positivity while those with posterior acoustic enhancement are likely to have negative receptor expression[14]. TNBC cancers were more likely to have circumscribed margins while hormone receptor positive cancers were more likely to show spiculated margins[15,16]. High risk microcalcifications detected on mammogram are associated with HER2-enriched cancers[17]. Our results are generally consistent with prior studies, with imaging features that correlate with the following subtypes: Spiculated margins were positively correlated with hormone receptor positive status (Luminal A or B). As hypothesized by earlier research, as luminal cancers tend to be lower grade and grow at a slower rate, they provoke a desmoplastic reaction, resulting in radiologic findings of spiculated margins. The desmoplastic reaction also affects acoustic impedance of the tumor to healthy tissue interface, causing excessive sonographic attenuation by the tumor, resulting in posterior shadowing[14,15,18].

High-risk microcalcifications, vascularity, and microlobulated margins, are positively associated with HER2 enriched cancers. Studies have shown that HER2 gene overexpression is linked to neo-angiogenesis *via* production of VEGF[19], while high-risk microcalcifications are due to the tendency of HER2 cancers to have a concomitant DCIS component[20]. Rapidly proliferating tumor cells, which consume the blood supply, lead to tumor necrosis and subsequent acidosis in the microenvironment, resulting in calcium accumulation in the ducts[21]. Our results concur with prior studies, which reported high risk microcalcifications to be more frequent in HER2+ tumors compared to luminal cancers[22].

A statistically significantly relationship exists between TNBC, which is the most aggressive molecular subtype, and posterior acoustic enhancement, circumscribed margins, and large tumor size. These are more cellular, grow rapidly, and do not generally incite a strong desmoplastic reaction from the surrounding healthy tissue[6]. The more regular interface between tumor and surrounding tissue probably results in a circumscribed margin, while internal necrosis and high cellularity probably attenuate the sound waves to a lesser degree, manifesting as posterior enhancement on

ultrasound. Concordant with other studies[8,11], US tumor size was a statistically significant predictor of TNBC cancers. In our sample, we found that if detected through breast screening (patients were asymptomatic), each unit increase in tumor size is associated with a higher odds ratio for TNBC cancer relative to the rest of the subtypes. This may imply that breast screening could be detecting TNBC at an earlier stage (smaller size, non-palpable), and that clinicians should prioritize biopsy of larger lumps when assessing screening populations.

DCIS is characterized by architectural distortions and high-risk microcalcifications on MG, but only 60% of DCIS tumors are visible on US (n = 31, 58%). High-risk microcalcifications tend to be absent in DCIS tumors that are sonographically visible. On US, a combination of small size and absence of posterior acoustic features favor DCIS. These two sonographic features are also commonly seen in benign lesions. However, similar to invasive cancers, the majority of sonographically visible DCIS show microlobulated or spiculated margins, whereas the overwhelming majority of benign lesions demonstrate circumscribed margins[23]. This highlights the importance of margin assessment of even small lesions on ultrasound, which depends on the skill of the performing technologist, as well as the use of a high-resolution probe.

In the current landscape of BC chemotherapeutic regimens, hormonal therapy is routinely used for hormone-receptor positive tumors (Luminal A/B), while targeted therapy, such as Herceptin, is available for cancers which overexpress HER2 (Luminal B/HER2-enriched). On the other hand, not only are TNBC tumors more aggressive (due to poor dedifferentiation), and have a higher recurrence rate, no targeted therapeutic strategy is currently available for the treatment of TNBC, leaving non-targeted chemotherapy as the only weapon in the chemotherapeutic arsenal. Stratification may allow for earlier resection times by aiding radiologists in deciding whether to biopsy or observe the lesion. As screening detects tumors at an earlier stage, large size is one discriminating feature that could point towards an aggressive TNBC subtype—prioritizing biopsy for these cases may benefit patient outcomes. In addition, histopathological analysis may not be readily available in certain developing countries, and an improved understanding of how the imaging features of BC correlate to molecular subtype would aid in tailoring the treatment strategy for patients who are cost constrained.

Our study had several limitations. First, as this was a retrospective study conducted at a single institution, it could have been subject to selection bias with respect to patients who arrive at our institution, even though we endeavored to minimize selection bias by including all consecutive patients within a fixed duration of time and blinding the assessing radiologists to the histology results. Second, due to the relatively small sample size, some of the subanalysis could have lacked statistical power to detect a significant difference in imaging features across molecular subtypes. Nevertheless, we see potential for further research, particularly in automated machine learning. From studies such as ours, imaging features identified as effective predictors could be used to customize deterministic algorithms for computer-extracted features, which could then be utilized at large-scale and with no inter-reader variability[2]. In fact, a recent study introduced a machine learning model for MRI classification of BC subtypes, guiding their radiomic feature selection and categorization by a review of the prior literature for imaging features that exhibited prognostic significance for various aspects of BC[24].

CONCLUSION

In conclusion, key features in mammographic and sonographic imaging were significantly associated with BC molecular subtypes. Knowledge of such correlations could help clinicians stratify BC patients according to their likely molecular subtype, potentially enabling earlier, more effective treatment or aiding in therapeutic decisions in countries where receptor testing is not readily available.

ARTICLE HIGHLIGHTS

Research background

There is evidence in the literature that breast cancer (BC) molecular subtypes often have characteristic imaging features on mammogram (MG), ultrasound (US) and magnetic resonance imaging. These imaging features on MG and US are of particular

interest as they are cost-effective and widely available even in many developing countries.

Research motivation

Thus far, research into the correlation between MG and US imaging features and BC subtypes has been based on populations of symptomatic patients, with the lack of data on an asymptomatic (screening) population highlighted as an area for future research. We wanted to thus use our data which consists of both screening and symptomatic patients to add to the body of knowledge on this issue. Also, our population includes patients with ductal carcinoma-in-situ (DCIS) which only a few papers have examined.

Research objectives

To correlate the MG and US imaging features with the molecular subtypes of BC (hormone receptor positive *vs* hormone receptor negative, triple-negative *vs* non-triple negative and HER2 positive *vs* HER2 negative) and DCIS in our population of screening and symptomatic patients.

Research methods

Our study is retrospective, with a population of 328 consecutive patients in 2017-18 with histologically confirmed BC. 237 (72%) were symptomatic, and 91 (28%) were detected *via* a screening program. All the patients underwent MG and US imaging prior to biopsy. The images were retrospectively interpreted by two breast-imaging radiologists with 5-10 years of experience who were blinded to the histology results to ensure statistical independence. To test the hypothesis that imaging features are correlated with tumor subtypes, univariate binomial and multinomial logistic regression models were performed. Also, multivariate logistic regression (with and without interaction terms) was utilized to identify combinations of MG and US imaging characteristics predictive of molecular subtypes.

Research results

Circumscribed margins, posterior enhancement, and large size are correlated with triple-negative BC (TNBC). High-risk microcalcifications and microlobulated margins is predictive of HER2-enriched cancers. DCIS is characterized by small size on US, absence of posterior acoustic features, and the presence of architectural distortion on MG. Hormone receptor positive subtypes tend to be small, with spiculated margins and posterior acoustic shadowing. These results are broadly consistent with findings from prior studies. In addition, we also find that US lesion size signals a higher odds ratio for TNBC if presented during screening.

Research conclusions

Several MG and US imaging features were shown to independently predict molecular subtypes of BC, in a population of both screening and symptomatic patients. Knowledge of such correlations could help clinicians stratify BC patients, possibly enabling earlier treatment for patients with triple negative cancer. This could also aid therapeutic decisions in countries where receptor testing is not readily available.

Research perspectives

To further research in this field, machine learning algorithms may be trained to recognize both the imaging characteristics as well as the radionomic characteristics of BC molecular subtypes, to see if this can further improve the predictive accuracy of imaging. More studies with asymptomatic populations of patients, and with differing ethnicities would also be useful to corroborate the results in our study.

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ORIGINAL ARTICLE

Retrospective Study

Features of primary pancreatic lymphoma: A bi-institutional review with an emphasis on typical and atypical imaging features

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Informed consent statement: As this is a retrospective review that involves no diagnostic or therapeutic intervention, as well as no direct patient contact, IRB permission was obtained with waiver of informed consent and waiver of authorization to use and

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Abstract

BACKGROUND

Primary pancreatic lymphoma (PPL) is a rare neoplasm. Being able to distinguish it from other pancreatic malignancies such as pancreatic ductal adenocarcinoma (PDAC) is important for appropriate management. Unlike PDAC, PPL is highly sensitive to chemotherapy and usually does not require surgery. Therefore, being able to identify PPL preoperatively will not only direct physicians towards the correct avenue of treatment, it will also avoid unnecessary surgical intervention.

AIM

To evaluate the typical and atypical multi-phasic computed tomography (CT) imaging features of PPL.

METHODS

A retrospective review was conducted of the clinical, radiological, and pathological records of all subjects with pathologically proven PPL who presented to our institutions between January 2000 and December 2020. Institutional review board approval was obtained for this investigation. The collected data were analyzed for subject demographics, clinical presentation, laboratory values, CT imaging features, and the treatment received. Presence of all CT imaging findings including size, site, morphology and imaging characteristics of PPL such as the presence or absence of nodal, vascular and ductal involvement in these subjects were recorded. Only those subjects who had a pre-treatment multiphasic CT of the abdomen were included in the study.

RESULTS

review patient information.

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Twenty-nine cases of PPL were diagnosed between January 2000 and December 2020 (mean age 66 years; 13 males/16 females). All twenty-nine subjects were symptomatic but only 4 of the 29 subjects (14%) had B symptoms. Obstructive jaundice occurred in 24% of subjects. Elevated lactate dehydrogenase was seen in 81% of cases, whereas elevated cancer antigen 19-9 levels were present in only 10% of cases for which levels were recorded. The vast majority (90%) of tumors involved the pancreatic head and uncinate process. Mean tumor size was 7.8 cm (range, 4.0-13.8 cm). PPL presented homogenous hypoenhancement on CT in 72% of cases. Small volume peripancreatic lymphadenopathy was seen in 28% of subjects. Tumors demonstrated encasement of superior mesenteric vessels in 69% of cases but vascular stenosis or occlusion only manifested in 5 out of the twenty-nine individuals (17%). Mild pancreatic duct dilatation was also infrequent and seen in only 17% of cases, whereas common bile duct (CBD) dilation was seen in 41% of subjects. Necrosis occurred in 10% of cases. Size did not impact the prevalence of pancreatic and CBD dilation, necrosis, or mesenteric root infiltration (P = 0.525, P = 0.294, P = 0.543, and P = 0.097, respectively). Pancreatic atrophy was not present in any of the subjects.

CONCLUSION

PPL is an uncommon diagnosis best made preoperatively to avoid unnecessary surgery and ensure adequate treatment. In addition to the typical CT findings of PPL, such as homogeneous hypoenhancement, absence of vascular stenosis and occlusion despite encasement, and peripancreatic lymphadenopathy, this study highlighted many less typical findings, including small volume necrosis and pancreatic and bile duct dilation.

Key Words: Pancreatic tumors; Primary pancreatic lymphoma; Pancreatic ductal adenocarcinoma; Imaging features; Diagnosis; Computed tomography

Core Tip: Primary pancreatic lymphoma (PPL) is often misdiagnosed as pancreatic adenocarcinoma. This two-center retrospective study of twenty-nine cases emphasized computed tomography (CT) imaging features useful for distinguishing PPL from its mimics. Distinct CT features of PPL, including a large homogenous hypovascular mass, absence of ductal dilation or atrophy, encasement of mesenteric vessels without invasion, and the presence of small volume peripancreatic adenopathy, should alert the clinicians to the potential diagnosis of this rare neoplasm. This study also demonstrated atypical findings such as necrosis and mild pancreatic or biliary ductal dilation should not rule out the diagnosis of PPL.

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INTRODUCTION

Primary pancreatic lymphoma (PPL) is an extremely rare malignancy, constituting less than 0.5% of pancreatic neoplasms and approximately 1% of all extranodal lymphomas[1-3]. The World Health Organization outlines PPL as a mass predominantly located in the pancreas with surrounding lymphatic node involvement[4]. In addition to these guidelines, Berhns' diagnostic criteria require (1) Normal white blood cell count; and (2) Nodal compromise defined within the peripancreatic region, without the presence of palpable superficial lymphadenopathy, mediastinal nodal enlargement, or hepatic and splenic involvement[5]. The clinical presentation of PPL often includes abdominal pain, weight loss, nausea and vomiting, and occasionally ob-

structive jaundice[4]. However, these nonspecific characteristics make it difficult to differentiate PPL from other pancreatic cancers, most notably the more common pancreatic ductal adenocarcinoma (PDAC)[2]. Furthermore, a paucity of information regarding PPL's distinctive radiologic findings makes diagnosis with imaging alone challenging[3].

Identifying unique preoperative characteristics of PPL so physicians may discriminate between PPL and PDAC early-on is crucial given their mutually exclusive prognoses and managements; while PDAC typically requires surgical intervention, the preferential treatment of PPL is chemotherapy with extensive follow-up due to this disease's high recurrence rate[3,4]. Therefore, this study aims to evaluate the common and uncommon computed tomography (CT) imaging appearances of PPL, as well as other diagnostic features that may enable definitive diagnosis.

MATERIALS AND METHODS

Study design

For this retrospective Health Insurance Portability and Accountability Act-compliant study, clinical, radiology, and pathology databases at the University of Texas MD Anderson Cancer Center, Houston, TX and Mayo Clinic, Phoenix, AZ were reviewed. This review searched the period between January 2000 and December 2020 for reports containing the strings "pancreas lymphoma" or "pancreatic lymphoma". Institutional review board permission was obtained for retrospective assessment of imaging, clinical, and pathologic data with waiver of informed consent from both the institutions involved in this study. The exclusion criteria were: (1) Lymphoma with mediastinal or pelvic adenopathy, bone marrow or hepatosplenic involvement which were considered to be systemic lymphoma and not PPL; (2) Pancreatic tumors suspected to be lymphoma on CT and later biopsy-proven to be another diagnosis on histological examination; (3) Subjects without a pretreatment multiphasic CT examination; and (4) Subjects whose medical records were not available of review.

The derivation of a cohort of 29 subjects is shown in Figure 1. All subjects had biopsy-proven PPL with a mass involving the pancreas. There were 13 males and 16 females with a mean age of 66 years (range, 55-88 years).

Imaging studies

Multislice CT scans of the abdomen were obtained in the pre-contrast, arterial, venous, and delayed phases. CT findings such as the size, site, morphology and imaging characteristics of PPL including the presence/absence of nodal, vascular and ductal involvement in these subjects were recorded by two reviewers (DG, KS) with 12 and 18 years of post-fellowship experience in Abdominal Imaging.

Statistical analysis

Descriptive statistics were compiled. Differences in sizes of tumors showing atypical findings (*i.e.,* pancreatic and bile duct dilation, mesenteric root infiltration, and tumor necrosis) and those without these findings was performed using the Mann-Whitney test. Frequency of these findings in tumors larger than 10 cm and less than 10 cm was performed with the Chi-Square test. MedCalc 19.3 (MedCalc Software Ltd, Ostend, Belgium) was used for statistical analysis.

RESULTS

Clinical presentation and management

The clinical presentation of the 29 biopsy-confirmed PPL cases used for imaging and clinical feature evaluation are summarized in Table 1. The most common presenting finding were vague abdominal pain (83%) and nausea and vomiting (31%). Weight loss was present in 21% of cases. Classic "B" symptom (*i.e.* fever, chills, night sweats, weight loss) were only seen in 14% of subjects. Similarly, 14% of cases demonstrated a palpable abdominal mass. Obstructive jaundice appeared in seven out of the twenty-nine subjects (24%).

All twenty-nine subjects underwent chemotherapy; the most common treatment was R-CHOP (given in 72% of cases). R-CVP was administered to 2 subjects (7%). In addition, 5 subjects (17%) underwent radiation therapy.

Table 1 Clinical presentation of primary pancreatic lymphoma

Characteristics	*n* (%)
Abdominal pain	24 (83)
Nausea and vomiting	9 (31)
Weight loss	6 (21)
Classic "B" symptoms	4 (14)
Palpable mass	4 (14)
Obstructive jaundice	7 (24)

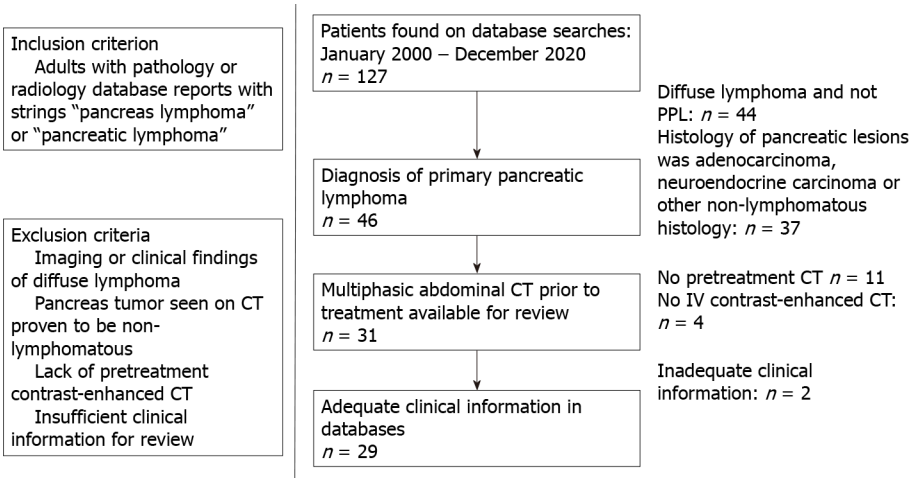

Figure 1 Inclusion and exclusion criteria of primary pancreatic lymphoma cohort. This figure gives the derivation of our cohort of 29 subjects for this study. CT: Computed tomography; PPL: Primary pancreatic lymphoma.

Laboratory findings

Lactate dehydrogenase (LDH) levels were elevated in 17 of the 21 cases for which LDH levels were recorded (81%). Elevated amylase was seen in four out of 11 subjects with recorded values (36%), all of whom presented with bile duct dilation (n = 3) or concomitant acute pancreatitis (n = 1). Cancer antigen 19-9 (CA 19-9) levels were within normal limits for 18 out of the 20 cases (90%). In the remaining two subjects biliary obstruction was present.

Imaging features

The prevalence of imaging findings are given in Table 2. The majority of tumors involved to the head and uncinate process (90%). Only four tumors (14%) occurred in the tail. Mean tumor size was 7.8 cm (range, 4.0-13.8 cm). Typical manifestation included a large (> 5 cm) infiltrative (52%) or focal, well-defined (48%) pancreatic mass, with homogenous hypoenhancement on post-contrast CT (72%) (Figure 2). In 41% of cases there was extension to the duodenum. Mass effect on the adjacent superior mesenteric vessels was seen in sixteen out of the twenty-nine subjects (55%). In addition, the majority of cases (69%) demonstrated encasement of superior mesenteric artery (SMA), superior mesenteric vein (SMV), or both; however, only five subjects (17%) had vascular stenosis or occlusion (Figure 3). In one subject there was active bleeding into the tumor by erosion of a mesenteric artery branch leading to intratumoral hematoma (Figure 4).

Furthermore, pancreatic ductal dilation was absent in 83% of cases; the remaining five subjects demonstrated mild dilation with duct caliber of less than 5 mm (Figures 3 and 5). There was no difference in size of those without and with pancreatic duct dilation (median sizes of 6.9 *vs* 6.8 cm, P = 0.525) or in the frequency of pancreatic duct dilation between tumors greater than 10 cm and less than 10 cm (P = 0.366). Common bile duct (CBD) dilation was seen in 41% of cases (Figure 3). Tumor size did not differ between those without and with CBD dilation (median sizes of 5.4 *vs* 7.1 cm, P = 0.294). There was no difference in frequency of CBD dilation between tumors greater

Table 2 Imaging features of primary pancreatic lymphoma

Characteristics	*n* (%)
Location	
Head	23 (80)
Body	3 (10)
Tail	4 (14)
Uncinate process	16 (55)
Mean size (cm)	7.8
Infiltrative mass	15 (52)
Focal mass	14 (48)
Homogeneous hypoenhancement	21 (72)
Tumor necrosis	3 (10)
Extension to nuodenum	12 (41)
Mass effect on SMV	16 (55)
Encasement of SMA/SMV	20 (69)
Vascular stenosis/occlusion	5 (17)
Pancreatic ductal dilation	5 (17)
Common bile duct dilation	12 (41)
Pancreatic atrophy	0 (0)
Infiltration of mesenteric root	4 (14)
Peripancreatic lymphadenopathy	8 (28)

SMV: Superior mesenteric vein; SMA: Superior mesenteric artery.

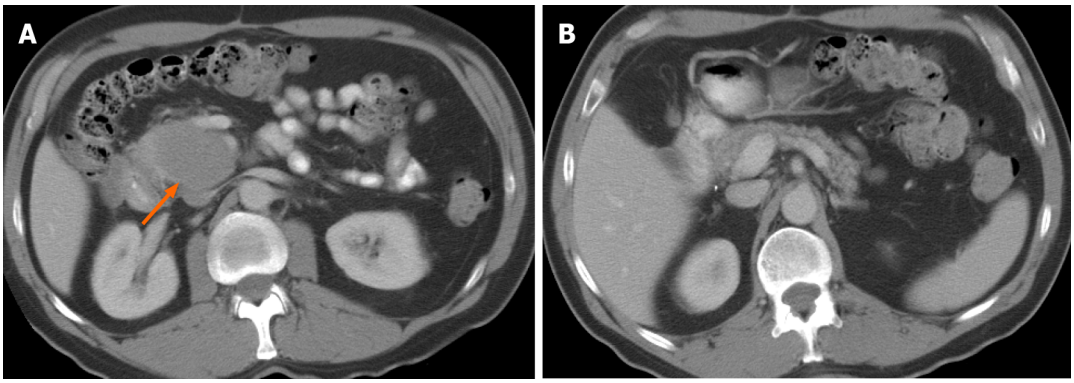

Figure 2 A 58-year-old male with abdominal pain. A: Axial computed tomography images demonstrate a large, relatively well-defined focal mass (arrow) involving the uncinate process of the pancreas; B: Axial computed tomography images. The tumor shows homogenous hypoenhancement, without evidence of any necrosis or calcification. The rest of the pancreas is preserved, and there is no pancreatic ductal dilation. Biopsy confirmed diagnosis of primary pancreatic lymphoma.

than 10 cm and less than 10 cm (P = 0.823)

No cases presented signs of pancreatic atrophy. Peripancreatic lymphadenopathy manifested in eight subjects (28%), five of which included involvement below the renal vein. These nodes all had a short-axis dimension of less than 1.5 cm. Mesenteric root infiltration was seen in 14% of cases (Figures 3 and 5). There was no significant difference in tumor size of those without and with mesenteric root infiltration (median sizes of 6.8 *vs* 12.8 cm, P = 0.097), although there was an overall trend for larger tumors to infiltrate the mesentery.

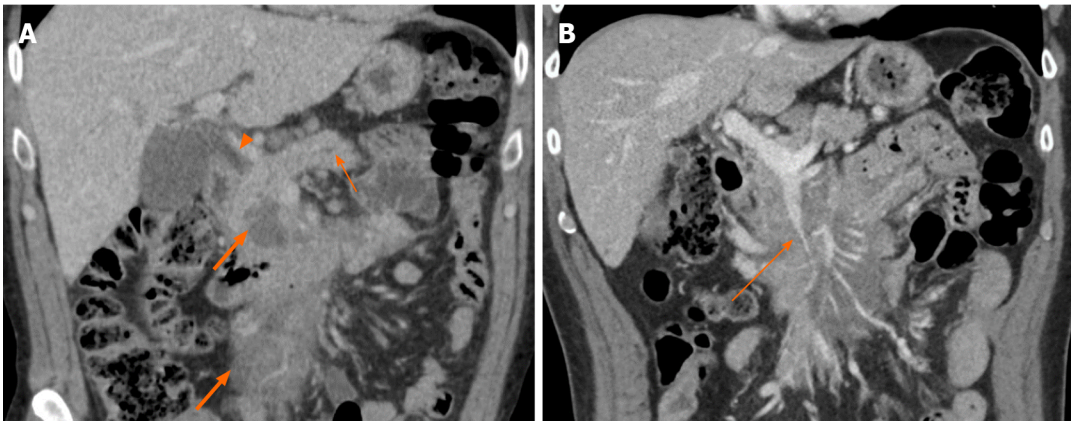

Figure 3 A 72-year-old male presenting with abdominal pain and jaundice. Coronal computed tomography imaging shows hypoenhancing homogenous infiltrative mass (thick arrows, A) infiltrating the mesenteric root. A: There is pancreatic duct dilation (short thin arrow) and common bile duct obstruction (arrowhead); B: There is stenosis of the superior mesenteric vein (long thin arrow) caused by the tumor.

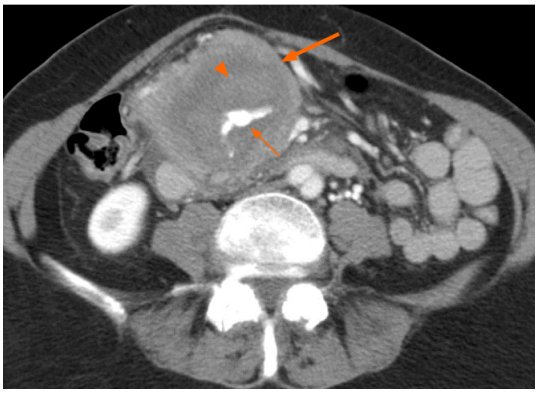

Figure 4 A 74-year-old female presenting with abdominal pain and nausea. Axial computed tomography shows mass (thick arrow) encircling an aneurysmal vessel (thin arrow) which is bleeding into the mass, causing hematoma (arrowhead). The tumor was biopsy-proven to be primary pancreatic lymphoma.

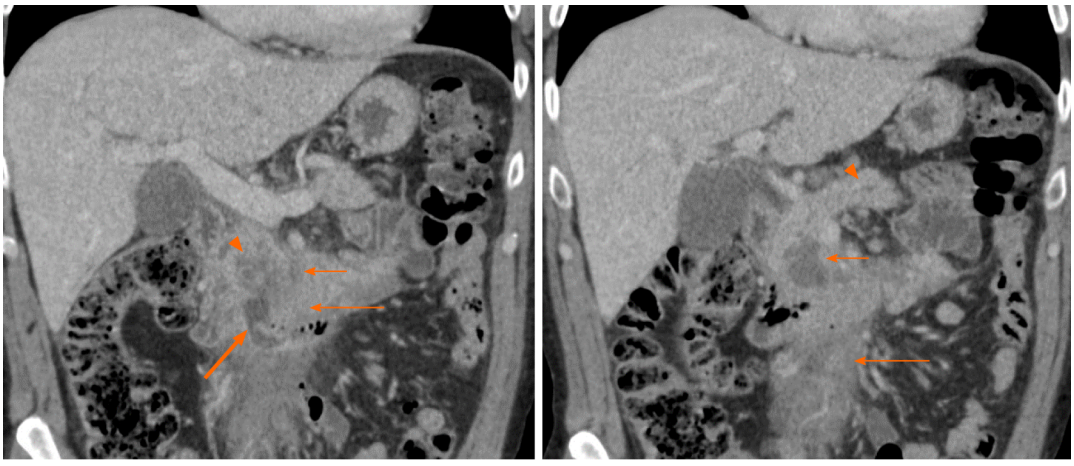

Figure 5 A 62-year-old male presenting with abdominal pain. Coronal computed tomography images show low-density necrosis (thin short arrow) within tumor (thin long arrow), which infiltrates the mesenteric root without occlusion of mesenteric vessels. There is a collection extending from the third part of the duodenum into tumor (thick arrow) suggesting fistula from probable erosion of duodenal wall. Mild pancreatic duct dilation is seen (arrowhead).

Small foci of low density within tumor less than 10% of volume, and likely to be necrosis, was seen in three cases (10%). These subjects either had acute pancreatitis (n = 2) or a duodenal fistula (n = 1) (Figure 5). There was no significant difference in size of those without and with necrosis (median sizes of 7.0 *vs* 6.2 cm, P = 0.543) or in frequency of necrosis between tumors greater than 10 cm and less than 10 cm (P =

0.950).

DISCUSSION

In agreement with prior literature, subjects in this study presented with nonspecific abdominal symptoms including vague epigastric pain, nausea and vomiting, weight loss, obstructive jaundice, and a palpable mass, making PPL difficult to distinguish from other pancreatic pathologies based on clinical presentation alone[4,6]. Out of these presentations, the most prevalent in PPL are abdominal pain, nausea and vomiting, and weight loss. The decreased prevalence of obstructive jaundice among PPL cases in comparison to individuals with PDAC could aid differentiating between the two; nevertheless, obstructive jaundice is still a relatively common potential presentation of PPL[7-10]. Classic "B" symptoms such as fever, chills, night sweats, and weight loss associated with systemic non-Hodgkin lymphoma are rare in PPL, and only occurred in 4 cases from this study[4].

Laboratory values may play a more decisive role in the diagnosis of the PPL. Elevated tumor marker CA 19-9 has been reported in 80% of PDAC cases but is a rare finding of PPL[4]. In this study, CA 19-9 was within normal limits for the vast majority of subjects. PPL cases with biliary dilation may be associated with mild elevation of CA 19-9[11]. In keeping with this study, elevated amylase has been observed in PPL by other studies and numerous case reports, often due to associated acute pancreatitis or biliary obstruction[8-10,12,13]. Other useful biomarkers include LDH which is often elevated in non-Hodgkin lymphomas, including PPL, but is less likely to be elevated in other pancreatic neoplasms such as PDAC[10,11].

Despite the potential use of laboratory values to suggest PPL over its mimics, the overall nonspecificity of clinical and laboratory features of PPL emphasizes the importance of imaging. CT is the most common imaging study performed for diagnosis and characterization of pancreatic masses[9]. PPL typically manifests as a diffusely infiltrative or focal, well-defined mass with homogenous hypoenhancement on post-contrast CT (Figure 2)[14,15]. In particular, the mass-like appearance can often be mistaken for PDAC. However, one CT feature that suggests PPL over PDAC is encasement of the SMA and/or SMV without narrowing or occlusion; conversely, locally advanced PDAC invades or stenoses these vessels[4,6]. In this study, 69% of cases demonstrated encasement of the SMA, SMV or both, but significantly less had vascular invasion or occlusion.

Lymphadenopathy localized to the peripancreatic region also suggests PPL[16]. In particular, the presence of lymphadenopathy below the level of renal hilum may be useful in excluding the diagnosis of PDAC[9,17,18]. The majority of cases in this study demonstrating lymphadenopathy presented nodal involvement below the renal hilum. In addition, all nodal involvement in this study was less than 1.5 cm in short-axis diameter, which may be useful for differentiating PPL from diffuse lymphoma secondarily involving the pancreas[19].

Perhaps the most important findings which indicate PPL rather than PDAC are the absence of pancreatic ductal dilation and pancreatic atrophy; only 17% of subjects in this study showed mild pancreatic ductal dilation (< 5 mm) and no subjects presented pancreatic atrophy (Figures 3 and 5)[14]. This is a useful differentiating feature as even small pancreatic adenocarcinoma located in the head of pancreas tends to result in moderate or severe upstream pancreatic ductal dilatation as well as pancreatic atrophy [4,6,20]. Nevertheless, mild pancreatic duct dilation may still be seen in PPL and should not be used to exclude this diagnosis.

In addition to cases of occasional mild pancreatic ductal dilation, this study has demonstrated multiple atypical features of PPL that have not been widely reported. Interestingly, CBD dilation was seen in a substantial proportion (41%) of subjects. While not widely reported in studies on PPL, Boninsegna *et al*[21] found similar results to this study, finding CBD dilation in six out of the fourteen subjects (43%) included in their series.

Ten percent of cases in this cohort showed partial necrosis of the tumor. This finding is contrary to the existing consensus that the presence of necrosis effectively excludes a diagnosis of PPL[5,7,11,17]. While most PPL do not present necrosis, this study demonstrates that complications such as concomitant acute pancreatitis or intratumoral fluid collection from a duodenal fistula may lead to this atypical finding (Figure 5).

To our knowledge, infiltration of the mesenteric root by PPL has not been reported by previous studies. This finding was demonstrated in 14% of cases in this study

(Figures 3 and 5). As expected, the mesenteric vessels were not thrombosed or occluded; however, in one case stenosis of the SMV was seen (Figure 3). There was active extravasation of blood into the tumor in one subject, likely as a result of tumor erosion of the vessels wall (Figure 4).

There are limitations of this study, including its retrospective design. In addition, PPL is a rare entity, and the combined databases of two major institutions only revealed 29 cases of biopsy-proven PPL with pretreatment multiphase CT. Nevertheless, this study revealed useful data on the differentiating features of PPL, and less typical findings which may occur.

CONCLUSION

PPL is a rare tumor, which may be misdiagnosed as PDAC, but differentiation between the two entities is critical in order to avoid unnecessary surgery and associated morbidity and mortality. CT imaging features, combined with clinical presentation and laboratory work, can help improve the diagnosis of PPL. The presence of a large relatively homogenous hypoenhancing mass involving the pancreatic head, absence of pancreatic ductal dilatation or atrophy, lack of vascular stenosis or occlusion despite encasement, and presence of small volume peripancreatic lymphadenopathy are helpful features for diagnosing PPL. However, unusual features such as tumor necrosis and the presence of mild pancreatic or bile duct dilation should not automatically exclude PPL as a potential diagnosis. It is important that clinicians are aware of both typical and atypical findings in order to raise the possibility of PPL without delay in diagnosis.

ARTICLE HIGHLIGHTS

Research background

Primary pancreatic lymphoma (PPL) is a rare neoplasm. The ability to differentiate PPL from other pancreatic malignancies including pancreatic ductal adenocarcinoma (PDAC) is important for appropriate management. However, the nonspecific characteristics currently associated with PPL and a lack of information regarding PPL's distinctive imaging features makes diagnosis difficult.

Research motivation

Identifying typical and atypical features of PPL on computed tomography (CT), as well as other diagnostic features that may differentiate PPL from its mimics, may enable definitive diagnosis. The discovery of features which distinguish PPL from PDAC early-on is critical to avoid unnecessary surgery.

Research objectives

This study aims to evaluate the typical and atypical CT imaging appearances of PPL. In addition, it distinguishes various clinical and laboratory markers which may be useful to identify PPL. An emphasis was placed on differentiating PPL from PDAC, which can be difficult to do using the current characteristics associated with PPL.

Research methods

Radiology, clinical, and pathology databases from two institutions were searched for reports between January 2000 and December 2020 containing the strings "pancreas lymphoma" or "pancreatic lymphoma". The exclusion criteria were: (1) Lymphoma with mediastinal or pelvic adenopathy, bone marrow or hepatosplenic involvement which were considered to be systemic lymphoma and not PPL; (2) Pancreatic tumors suspected to be lymphoma on CT and later biopsy-proven to be another diagnosis on histological examination; (3) Subjects without a pretreatment multiphasic CT examination; and (4) Subjects whose medical records were not available of review. Multislice CT scans of the abdomen were viewed in the pre-contrast, arterial, venous, and delayed phases. The clinical presentation, management, laboratory findings, and imaging features of all included patients (n = 29) were evaluated.

Research results

All twenty-nine subjects were symptomatic, but only 14% demonstrated B symptoms

and 24% demonstrated obstructive jaundice. Lactate dehydrogenase (LDH) levels were elevated in 17 of the 21 cases for which LDH levels were recorded (81%), however cancer antigen 19-9 (CA 19-9) levels were within normal limits for 18 out of the 20 cases for which values were recorded (90%). Pancreatic ductal dilation was absent in 83% of cases and no patients presented pancreatic atrophy. Atypical features of PPL included pancreatic bile duct dilation (17%), common bile duct (CBD) dilation (41%), necrosis (10%), and infiltration of the mesenteric root (14%). Size did not impact the prevalence of pancreatic and CBD dilation, necrosis, or mesenteric root infiltration (P = 0.525, P = 0.294, P = 0.543, and P = 0.097, respectively).

Research conclusions

The decreased prevalence of obstructive jaundice, elevated CA 19-9 levels, pancreatic ductal dilation, and pancreatic atrophy, as well as the increased elevation of LDH levels, encasement of the small mesenteric artery and/or vein without invasion or stenosis, and lymphadenopathy limited to the peripancreatic region, may be useful for distinguish PPL from its mimics, such as PDAC. However, in addition to the occasional appearance of pancreatic ductal dilation, this study identified multiple atypical features of PPL including the presence of CBD dilation, necrosis, and infiltration of the mesenteric root.

Research perspectives

Prospective studies with larger cohorts must be conducted to support the findings of this paper and the potential use of its highlighted imaging and clinical features for definitive diagnosis of PPL. In addition, there is a need for direct comparison of the frequency of these features in PPL *vs* PDAC, to determine how useful they are in differentiating the two entities.

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