Application of Molecular Biology to Individualize Therapy for Patients with Liposarcoma

Gulam Abbas Manji, MD, PhD, Samuel Singer, MD, Andrew Koff, PhD, and Gary K. Schwartz, MD

OVERVIEW

Liposarcomas are one the most common of over 50 histologic subtypes of soft tissue sarcomas that are mostly resistant to chemotherapy. Histologically, liposarcomas themselves are heterogeneous and fall into four distinct subtypes: well-differentiated/atypical lipomatous tumor, dedifferentiated liposarcoma, myxoid (round cell) liposarcoma, and pleomorphic liposarcoma. Surgical resection with negative margins remains the mainstay for definitive treatment for operable disease. For unresectable disease, retrospective studies have identified myxoid (round cell) and pleomorphic sarcomas to be relatively responsive to chemotherapy. Recent studies have identified distinct genetic aberrations that not only aid in the diagnosis of particular liposarcoma subtypes, but represent actionable targets as they are considered central to disease pathogenesis. Cyclin-dependent kinase 4 (CDK4) and murine double minute 2 (MDM2) are overexpressed in well-differentiated and dedifferentiated liposarcomas and offer tantalizing opportunities that are being pursued in clinical trials. Myxoid (round cell) liposarcomas appear to be sensitive to trabectedin, which is currently under U.S. Food and Drug Administration (FDA) review. Liposarcomas do not represent a uniform disease and understanding the underlying molecular mechanism will help not only in accurate diagnosis but in selecting the appropriate treatment.

Soft tissue sarcomas (STS) are a complex heterogeneous collection of more than 50 neoplasms of mesenchymal origin. According to the American Cancer Society, in 2015 approximately 11,930 patients in the United States will be diagnosed with STS, and nearly 4,870 will die from the disease. Surgery is the mainstay of therapy. For metastatic STS, cytotoxic chemotherapeutic agents such as doxorubicin or gemcitabine plus docetaxel have been used with response rates of approximately 25%, and overall survival (OS) of 12 to 18 months.1,2 Whenever possible, treatments are now tailored to the histologic subtype, tumor characteristics, molecular signature, and patient performance status. Liposarcoma is among the most common sarcoma subtypes, with average annual, age-adjusted incidence of 0.4 to 1.1 per 100,000 persons dependent on gender and race.3 Liposarcomas are a heterogeneous group of adipocytic neoplasms that are generally resistant to chemotherapy. This review will focus on management of liposarcomas and discuss novel targets and therapies directed toward them that are being tested in clinical trials.

LIPOSARCOMAS

Liposarcomas are neoplasms of adipocytes that normally grow slowly and present as a painless nonulcerated enlarging mass. They are subclassified into distinct categories dependent on their histology, molecular signature, and behavior:4

- Well-differentiated/atypical lipomatous tumor
- Dedifferentiated liposarcoma
- Myxoid (round cell) liposarcoma
- Pleomorphic liposarcoma
- Liposarcoma, not otherwise specified

Rarely, a specimen may have a combination of morphologic subtypes; this is referred to as mixed liposarcoma.

ATYPICAL LIPOSOMATOUS TUMOR/WELL-DIFFERENTIATED LIPOSARCOMA

Atypical lipomatous tumor (ALT) or well-differentiated liposarcoma (WDLS) is the least aggressive of the malignant forms of liposarcoma and represents 40% to 45% of the liposarcomas that are diagnosed.5 Microscopically, they consist of scattered lipoblasts with a single atypical nucleus surrounded by large intracytoplasmic vacuoles in a background of adipocytes.4 ALT/WDLS are usually indolent and tend not to metastasize, but can recur locally. However, if they undifferentiate into the dedifferentiated form, they exhibit an aggressive phenotype and are likely to metastasize.6 The retroperitoneum and extremities are common primary sites, but they can also occur within the mediastinum and parates-

From the Division of Hematology and Oncology, Columbia University School of Medicine, Herbert Irving Comprehensive Cancer Center, New York, NY; Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY; Program in Molecular Biology, Memorial Sloan Kettering Cancer Center, New York, NY.

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Corresponding author: Gary K. Schwartz, MD, Division of Hematology and Oncology, Columbia University School of Medicine, 177 Fort Washington Ave., Ste. 6-435, New York, NY 10032; email: schwartzg@columbia.edu.

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Dedifferentiated liposarcoma (DDLS) is the second most common type of liposarcoma diagnosed. DDLS is cellular and consists of nonlipogenic sarcoma that transitions abruptly within an ALT.4 DDLS has morphologic and molecular similarities to ALT/WDLS but behaves aggressively. DDLS has a higher rate of recurrence, with 20% to 30% distant metastasis, and a 6-fold higher risk of death compared to ALT/WDLS.8,9 Many of the gene alterations found in WDLS are also found in DDLS.7 For example, both CDK4 and MDM2 are overexpressed in both of these diseases. Genomic alterations of 207 STS, which included 50 DDLS specimens, confirmed the prior findings of amplifications within 12q12–15, including CDK4 and MDM2.10 WDLS and DDLS respond very poorly to chemotherapy and new treatment options are desperately needed.11

CDK4 and MDM2 Inhibitors

CDK4—Palbociclib. CDK4 is amplified in more than 90% of WDLS and DDLS cases, making it an opportune target in this disease subtypes. Preclinically, palbociclib (PD0332991), a CDK4/6 inhibitor, induces a G1 cell cycle arrest in liposarcoma cell lines that overexpress CDK4.10 A phase I trial with palbociclib in patients with advanced solid malignancies identified one patient each with ATL/WDLS and DDLS who had stable disease lasting several years at a dose of 200 mg daily for 14 consecutive days in 21-day cycles.12 This led to an open-label phase II trial for patients with WDLS and DDLS. Patients’ tumors were required to have CDK4 amplification and retinoblastoma protein (RB) expression, which is the downstream target for CDK4. Preclinically, RB expression was required for palbociclib activity.10 Among the 30 patients enrolled (five with WDLS and 25 with DDLS), the trial reported a 12-week progression-free survival (PFS) rate of 66%, surpassing the primary endpoint criteria of 40%. Significant hematologic toxicities were observed, including grade 3 and 4 anemia (17%), thrombocytopenia (30%), neutropenia (50%), and febrile neutropenia (3%).13 The results of a phase II study with a modified oral dose of 125 mg daily for 21 consecutive days in 28-day cycles (NCT01209598) were reported at the 2013 ASCO Annual Meeting, which indicated a comparable 12-week PFS but with reduced bone marrow toxicity.14

CDK4—LEE001 and LY2835219. Other CDK4 inhibitors are in clinical trials, including LEE001 and LY2835219. These agents exhibit a somewhat different toxicity profile, with neutropenia and QTc prolongation more common with LEE001 and gastrointestinal toxicity more common with LY2835219.15 Stable disease was noted in patients with liposarcoma, although the complete results with these agents in this disease are pending publication.

One unresolved question is whether there exists a biomarker to predict clinical benefit. Rb expression appears to be necessary, but its presence is not predictive of benefit.

MDM2 targeting. Recently, it has been shown that CDK4 inhibition induces a G1 cell cycle arrest in all liposarcoma cells. However, only some of these cells undergo a process of cell death called cellular senescence. The proteolytic turnover of MDM2 appears critical for the induction of cellular senescence induced by palbociclib and by suppression of CDK4 by siRNA. Furthermore, the loss of MDM2 expression induced by CDK4 inhibition appears to be dependent on the baseline expression of ATRX. An examination of MDM2 loss in liposarcoma patients treated with palbociclib has been shown to correlate with prolonged stable disease; whereas patients with no change in MDM2 expression had rapid disease progression with this therapy. The importance of baseline ATRX expression, and the loss of MDM2 expression as a marker of clinical benefit to CDK4 inhibitors in liposarcoma (as well as other tumor types), will require validation in future clinical trials.17

MDM2 gene amplification or protein overexpression has been demonstrated in several cancer types, including DDLS.16 Disruption of MDM2 and p53 interaction in cancer types with wild-type p53 and MDM2 overexpression has shown promise in vitro and in vivo. Nutlins are a class of imidazoline compounds that have been shown to have potent and selective activity against MDM2.19 In preclinical studies, nutlin-3a was the first drug to show potent in vitro effects in liposarcoma cell lines with amplifications and overexpression of MDM2.7 This drug induced both cell

**KEY POINTS**

- Sarcomas are rare malignancies of mesenchymal origin that represent a heterogeneous group of over 50 subtypes, of which liposarcomas are one of the most common subtype.
- Liposarcomas contain at least four distinct subtypes for which treatment has to be tailored.
- Well-differentiated liposarcoma is the least malignant form, does not respond to chemotherapy, and tends to recur locally.
- Dedifferentiated liposarcoma has some response to chemotherapy and, like well-differentiated liposarcoma, contains amplification/overexpression of murine double minute 2 and cyclin-dependent kinase 4, inhibition of which have shown promise in early-phase clinical trials.
- Retrospective data indicate trabectedin to have activity in myxoid (round cell) liposarcoma, which is currently under U.S. Food and Drug Administration review for this indication.
cycle arrest and apoptosis in these cells, which were also p53 wild-type.¹⁹

MDM2—RG7112. RG7112 was the first nutlin inhibitor to be tested clinically. In a proof-of-concept study, 20 chemotherapy-naïve patients with primary or relapsed WDLS (11 patients) or DDLS (9 patients) were enrolled in the neoadjuvant setting. Patients were treated with up to three cycles with 1,440 mg/m² of RG7112 for 10 days on a 28-day cycle schedule. Two of the 20 patients carried missense mutations in TP53 and 14 of 17 had MDM2 amplifications (three did not have adequate samples). Patients underwent paired biopsies before and on day 8 of treatment. p21 expression, a marker of p53 function, increased 3.48-fold in tumor tissue as measured by immunohistochemistry, while macrophage inhibitory cytokine-1 (MIC-1) levels, a marker of apoptosis and p53 induction, increased in blood. p53 expression and MDM2 transcripts also increased by 4.86- and 3.03-fold compared to baseline, respectively. Nineteen patients received at least one cycle of RG7112, of which ten and five completed three and two cycles, respectively. One patient achieved a partial response, 14 had stable disease, and five experienced disease progression (all of whom had DDLS).²⁰ Ten patients underwent surgical resection at a median of 34.5 days after the last dose of RG7112, of which eight had a complete resection and two had partial resections.²⁰ Patients with partial resection received three cycles of adjuvant treatment and maintained stable disease.

Ten serious treatment-related adverse events (AEs) were observed in seven patients, all of which were hematologic in nature. Grade 3 or 4 AEs included neutropenia (30%), febrile neutropenia (5%), thrombocytopenia (15%), nausea (5%), emesis (10%), diarrhea (5%), and general deterioration (5%). One patient died of nontreatment-related postoperative hemorrhagic complications. Hematologic AEs, including thrombocytopenia, led to treatment delays in many patients; three had to discontinue treatment. All patients recovered from their hematologic AEs and there were no treatment-related bleeding complications.²⁰ RG7112 treatment in mice and monkeys also resulted in thrombocytopenia by promoting megakaryocyte progenitor cell destruction by apoptosis and inhibition of DNA synthesis during endomitosis, a key step for platelet production.²¹

MDM2—SAR405838. SAR405838 is an oral spirooxindole derivative antagonist that binds to MDM2 with a Ki of 0.88 nmol/L, has high specificity, and has a dissociation constant of 9.4 nmol/L. A cocrystal structure of SAR405838 and MDM2 indicates that SAR405838 mimics three key p53 amino acids and binds to the unstructured N-terminus of MDM2 to achieve high affinity.²² SAR405838 activated wild-type p53 function in vitro and in xenograft tumor tissue of solid tumors, leading to p53-dependent cell cycle arrest and/or apoptosis. SAR405838 resulted in transcriptional up-regulation of PUMA, a transcriptional target of p53, induction of apoptosis, and complete tumor regression in SJSA-1 osteosarcoma xenograft model.²² A first-in-human phase I trial in patients with solid tumors evaluated once-daily and weekly schedules of SAR405838.²³ Only patients with DDLS were included in the maximum tolerated dose (MTD) expansion cohort. Sixty-eight patients were enrolled in the trial, of which 43% had liposarcoma. At the time of publication, 65 patients had discontinued study participation and three remained on study. Of the 65 patients, 47 discontinued participation because of disease progression, nine because of AEs, and nine for other reasons. Median duration of treatment was 42 days and MTD was established at 300 mg orally daily. Within the expansion cohort, 21 patients with DDLS were treated at 300 mg orally daily. The most common treatment-related AEs within this cohort included nausea (38%), fatigue (33%), diarrhea (24%), and thrombocytopenia (14%). Eight patients discontinued treatment because of AEs. Of note, no dose-limiting toxicities were observed on the weekly schedule of SAR405838.²³ Pharmacokinetic studies indicated a Tₘₚ₅ of approximately 4 hours and half-life of nearly 16 hours. Plasma MIC-1 protein concentration correlated positively with SAR405838 exposure. Interestingly, a lower platelet count correlated with higher SAR405838 levels on a daily administered schedule, but a higher Cₘₚ₅ achieved on the weekly schedule did not correlate with lower platelet counts. Overall, the best response was stable disease in 32 patients (47%), of which 13 patients (19.1%) had stable disease at 12 weeks. In the MTD expansion cohort with DDLS, 11 of 21 patients (52%) had stable disease as best response.²³
MYXOID (ROUND CELL) LIPOSARCOMA

Myxoid (round cell) liposarcomas (MRCL) mostly arise in deep soft tissue of the extremities in patients who tend to be younger than those with other sarcoma subtypes. MRCL represents a single entity with variable round cell component and accounts for 30% to 35% of liposarcomas.²⁴ Round cell liposarcoma is considered a more aggressive subtype of myxoid liposarcoma, with greater than 5% round cell component carrying an unfavorable prognosis.²⁵,²⁶ Unlike other tumors, MRCL tends to spread to serosal surfaces, bones, the abdominal cavity, and other soft tissues, even in the absence of lung disease.²⁷ Microscopic evaluation identifies tumors composed of uniform round-to-oval primitive nonmesenchymal cells and a variable number of monovacuolated lipoblasts. More than 90% of MRCL tumors contain the 12q13 and 16p11 translocation that leads to the FUS-DDIT3 (TLS-CHOP) fusion. Fused in sarcoma (FUS) protein, EWSR1, and TAF15 belong to the FET family of proteins and are involved in regulation of transcription and RNA splicing. DDIT3 is a leucine zipper containing transcription factor and is a member of the C/EBP family that plays a role in adipocyte differentiation and cell cycle control.²⁸ The fusion protein is thought to result in malignant transformation by favoring adipocyte differentiation over proliferation. These tumors are also characterized by mutations in PIK3CA, encoding the catalytic subunit of phosphatidylinositol 3-kinase (PI3K).¹⁰ In a series of 71 patients with MRCL, 13 had point mutations in PIK3CA. These mutations were clustered in two domains, the helical domain (E542K and E545K) and the kinase domain (H1047L and H1047R). Interestingly, patients whose tumors harbored mutations in PIK3CA had a shorter duration of disease-specific survival than those with wild-type PIK3CA (p = 0.036, log-rank test).¹⁰

Surgery alone or with radiation therapy is the mainstay of treatment, but despite appropriate treatment for local disease, nearly 40% of patients experience disease relapse.²⁹ Although MRCL is relatively more chemotherapy-sensitive compared to other STS, median survival is 2 years in the advanced disease setting.³⁰

Trabectedin

Apart from chemotherapy, trabectedin (ET-743), a marine alkaloid, is approved in Europe as second-line treatment for advanced STS and is a promising agent, particularly in MRCL. Trabectedin has two known functions: 1) It binds to DNA within the minor groove of the double helix, causing a conformational change that likely changes its interactions with DNA-binding proteins, and 2) it causes double-stranded DNA breaks by interaction with transcription-coupled nucleotide excision repair complex. Owing to its ability to alter DNA–protein interaction, it is not surprising that trabectedin specifically inhibited type I and II TLS-CHOP transcripts from binding to their cognate target promoters in an MRCL xenograft model that impeded TLS-CHOP function.³¹ Recent evidence has suggested that trabectedin also affects the tumor microenvironment by targeting monocytes and macrophages that have protumoral functions, including production of growth factors, neoangiogenesis, increased protease activity, and support in tumor cell dissemination.³²-³⁵

Fifty-one patients with advanced myxoid liposarcoma treated with trabectedin on a compassionate-use basis were analyzed retrospectively and found to have an overall response of 51%, including two patients who achieved a complete response. Median PFS was 14 months and PFS at 6 months was 88%.²⁴ In a single-center retrospective study of 21 patients treated with trabectedin for a median of four cycles, an objective partial response was achieved in three patients (14%) and eight patients (38%) achieved stable disease for a median duration of 4.5 months.³⁶ The benefit of trabectedin in other liposarcoma subtypes remains to be defined.

PLEOMORPHIC LIPOSARCOMA

Pleomorphic liposarcoma (PLS) represents approximately 5% to 15% of liposarcomas³⁷ and usually presents in adults within the lower extremity.³⁸ PLS pathologically exhibits large, multivacuolated pleomorphic lipoblasts.³⁹ PLS is associated with a poor prognosis, with high local recurrence and distant metastasis in the range of 30% to 35%.⁴⁰ Because of the rarity of the disease, treatment data within this particular subtype is limited. A retrospective study reviewed response to chemotherapy in 39 patients with unresectable or metastatic PLS.⁴¹ Of the 32 patients assessable for response, one (3%) had a complete response, 11 (34.5%) had a partial response, and nine (28%) had stable disease, with a median follow-up of 62 months. The median PFS and OS were 4.3 and 14 months, respectively. The overall objective response rate was 37%. Interestingly, anthracycline-based regimens did not result in an improved response rate compared to nonanthracycline-based therapies.⁴¹

CHEMOTHERAPY AND LIPOSARCOMA SUBTYPE SENSITIVITY

Overall, liposarcomas do not respond well to systemic cytotoxic chemotherapy. The Royal Marsden study addressed the subtype-specific sensitivity to chemotherapy in a retrospective analysis of a prospectively maintained database of patients treated between August 1989 and June 2004. Of the 88 patients with liposarcoma treated, 94% of patients received first-line chemotherapy for metastatic disease or for local recurrence. Thirty percent received doxorubicin and 17% received ifosfamide as monotherapy, while 34% received the combination. Of the subtypes treated, the most response rates were observed in the myxoid subtype (48%), while PLS (33%), DDLS (25%), and round cell liposarcoma (17%) achieved some response, in contrast to WDLS which had none. Combination chemotherapy with doxorubicin and ifosfamide resulted in the greatest response rates across subtypes.

Another large retrospective study of 208 patients, which focused on patients with advanced WDLS and DDLS from 11 participating institutions, identified that 85 patients (41%)
received combination chemotherapy and 123 patients (59%) received single-agent chemotherapy. The majority of patients (82%) received anthracycline-based therapy. Only 21 patients (12%) achieved an objective response, all of whom received anthracyclines. With a median follow-up of 28 months, median PFS and OS were 4.6 and 15.2 months, respectively. A trend toward a higher median PFS in WDLS (8.7 months) compared to DDLS (4 months) was noted. On multivariate analysis, age and performance status (PS) were the sole factors that independently associated with PFS, while grade and PS associated with OS.

**SUMMARY AND FUTURE DIRECTIONS**

Although surgical resection remains the cornerstone for localized disease, treatment for unresectable or metastatic disease is highly dependent on the liposarcoma subtype. For patients with rapidly growing WDLS or DDLS, doxorubicin-based regimens can be considered as a treatment option, although response rates remain low. In contrast, for patients with MRCL, doxorubicin-based chemotherapy is the treatment of choice and trabectedin (where available) can also be considered as a treatment option. The role of PIK3CA inhibitors in this disease remains to be defined, but the opening of MATCH trials through the National Cancer Institute with agents targeting PIK3CA may soon answer this question. The importance of trabectedin and newer cytotoxic agents, such as eribulin in liposarcoma subtypes other than MRCL, continues to be defined. For PLS, chemotherapy remains the treatment of choice, although the prognosis in this setting is poor. Palbociclib, a CDK4/6 inhibitor, which was recently approved in combination with letrozole for patients with es-

**References**


