Вестник РУДН. Серия: МЕДИЦИНА
RUDN Journal of MEDICINE. ISSN 2313-0245 (Print). ISSN 2313-0261 (Online)
2023;27(4)
http://journals.rudn.ru/medicine

DOI: 10.22363/2313–0245–2023–27–4–459–469
EDN: ITZWHG

REVIEW
ОБЗОРНАЯ СТАТЬЯ

Experimental models of tumor growth in soft tissue sarcomas

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Abstract. Soft tissue sarcomas are rare tumors (about 1 % of all malignant neoplasms) and include more than 70 histological subtypes, the pathogenetic features of which remain unclear. This is largely due to both quantity and volume of clinical material and high heterogeneity of the disease. Given the rarity and heterogeneity of each individual subtype of soft tissue sarcoma, there is an urgent need to develop universal model systems to understand the molecular changes that determine tumor biology. Such systems include CDX models (cell line-derived xenograft), created from cell lines, PDX (patient-derived xenograft), obtained from primary tumor/metastasis cells, both a whole fragment of surgical material and from a cell suspension; humanized animals containing various human immune cells, and GEM (genetically engineered mouse) models, which are created through transfection of genetic changes characteristic of different subtypes of soft tissue sarcomas. To create these systems, not only widely available mouse models are used, but also other animals, such as fish *(Danio rerio)*, rats, pigs, and dogs. Another important goal of using animal models is to screen the effectiveness of modern drugs. To date, treatment of various subtypes of soft tissue sarcomas is based on standard protocols of chemotherapy (doxorubicin, epirubicin, dacarbazine, ifosfamide) and surgical resection. In the case of inoperable forms or late stages of soft tissue sarcomas, animal models are a potential tool in predicting the effectiveness of therapy and personalized selection of treatment regimens. In this regard, studies of the mechanisms of targeted action on specific molecules and the use of humanized animals for the development of new approaches to immunotherapy are of particular relevance. The current review discusses animal model systems of the three most common types of soft tissue sarcomas: liposarcomas, undifferentiated pleomorphic and synovial sarcomas, as well as the use of these models to find new therapeutic solutions. *Conclusion.* Currently, PDX and GEM models are widely used to identify molecules and signaling pathways involved in the development of sarcomas, identify tumor-initiating cells, and assess the chemoresistance of known drugs and new drugs at the level of the entire tumor ecosystem. However, the key problems of animal models of soft tissue sarcomas remain changes in their composition and phenotype compared to the original tumor, poor survival of surgical material, and lack of cellular immunity in immunocompetent models, high cost, and the length of time it takes to create and maintain the model. A solution to one of the problems may be the use of humanized animals with PDX, which implies the presence in the model of immune, stromal and tumor components that are as close as possible to the human body.

Keywords: soft tissue sarcomas, *in vivo* models, therapy

Funding. This study was supported by the Russian Science Foundation (grant 23–65–00003).

Author contributions. All authors have made significant contributions to the concepts, manuscript preparation, read and approved final version before publication.

Conflicts of interest statement. The authors declare no conflict of interest.

Acknowledgements — not applicable.

Ethics approval — not applicable.

Consent for publication — not applicable.

Received 12.10.2023. Accepted 14.11.2023.

For citation: Tretyakova MS, Bokova UA, Korobeynikova AA, Denisov EV. Experimental models of tumor growth in soft tissue sarcomas. *RUDN Journal of Medicine.* 2023;27(4):459–469. doi: 10.22363/2313–0245–2023–27–4–459–469

Introduction

Sarcoma is a rare tissue disease of mesenchymal origin that forms in bones, adipose tissue, joints and muscles and is divided into two main groups: soft tissue sarcomas (STS) (more than 70 types) and bone sarcomas (osteosarcoma, chondrosarcoma and Ewing sarcoma) [1]. The biology of sarcomas remains poorly understood due to high heterogeneity, different origins and histology, but a common feature is a poor prognosis in patients with advanced disease [2].

Given the rarity of each individual subtype of STS and the heterogeneity of the disease, there is an urgent need to develop model systems to understand the molecular changes that determine tumor biology, diagnosis, prognosis and the effectiveness of disease therapy. However, the limited number of model systems available in oncology makes the selection of suitable models even more challenging. Well-known mouse models are successfully used in preclinical studies of new therapeutic agents and selection of therapy for various oncological diseases [3–9]. Therefore, it is advisable to use these models to study various subtypes of sarcomas as well. In addition to the widely available mouse models, other animals, such as *Danio rerio* fish, rats, pigs and dogs, can serve as platforms for testing hypotheses about genetic factors contributing to the initiation and/or progression of cancer and, to a sufficient extent, reflect intertumor heterogeneity [10–13]. Thus, despite high heterogeneity and low incidence of STS, in recent decades various biological systems have been developed to model the disease in order to identify pathogenetically significant signaling pathways, mutations and markers and to develop new methods of antitumor therapy.

STS *in vivo* models

Information on the population composition of STS is mainly obtained from *in vitro* studies [14, 15], which use various cell lines: primary, immortalized and 3D cultures [16–18]. However, it is well known that when cells adapt to artificial culture conditions, they proliferate faster than parental tumor cells, acquiring new phenotypic characteristics that change their characteristics and therapeutic response [14]. Therefore, it is more expedient to study tumor cells biology and evaluate treatment effectiveness in a living organism.

Modeling a tumor disease *in vivo* involves methods of engrafting tumor cells into laboratory animals. Such models are divided into 2 types: «cell line-derived xenograft» (CDX) — a xenograft obtained from a cell line, and «patient-derived xenograft» (PDX) — a model obtained from primary tumor/metastasis cells. Both models are representative and predictive for basic and

translational research [19, 20]. Although CDX is still the most commonly used model due to its wide availability and ease of use, PDX is the most effective in terms of translational potential.

Another approach to modeling sarcomas *in vivo*, when tumor cell transplantation is not necessary, is to induce *de novo* sarcomagenesis in immunocompetent mice. Such environmentally induced (EIM) and genetically engineered mouse (GEM) models are applicable to specific subtypes of STS, but their development is complex and time-consuming. EIM models are obtained by exposure to various physical factors (for example, intramuscular injection of cardiotoxin and barium chloride), while GEM models are created through transfection of genetic changes characteristic of sarcomas [21, 22]. These model systems have made it possible to expand knowledge about oncogenic, tumor suppressor and other signaling pathways associated with the development of sarcomas.

Experimental models of liposarcomas

Liposarcomas are tumors of adipose tissue and are divided into several subtypes, among which the common ones are well-differentiated and dedifferentiated liposarcomas. The diagnosis of each subtype is based on anatomical location, clinical presentation and histology, and is characterized by a distinctive set of genetic features [23, 24]. However, well-differentiated and dedifferentiated liposarcoma may represent the same subtype, since both are associated with amplification in the chromosomal region 12q13–15, which causes overexpression of the *MDM2* and *CDK4* genes [25].

A well-differentiated liposarcoma has a low incidence of metastasis and indolent course and can be considered a low-grade tumor, while other subtypes of liposarcoma demonstrate a high metastatic potential [25]. Liposarcomas vary in location, but most often they are observed in the retroperitoneum. This pattern facilitates modeling *in vivo*, since the introduction of tumor cells into the abdominal cavity is a routine method for obtaining intraperitoneal tumors [26].

Typically, when modeling liposarcomas from the primary tumor, samples are dissociated into a homogeneous cell suspension for the purpose of injection into immunocompromised mice [27]. The immunocompetent mouse model (nonobese diabetic/severe combined immunodeficiency, NOD/SCID) has a weakened immune system and is considered the most effective for xenotransplantation. The tumor suspension can be administered subcutaneously (ectopic xenograft) or into a specific organ (heterotopic model). Several cases of obtaining PDX models have been described, including for selecting individual treatment for patients with liposarcoma. The development of such models usually takes a long time, up to six months, and up to 75 % of implanted tumors successfully assimilate in animal organisms [28]. PDX models of liposarcomas make it possible to select personalized therapy, achieving high efficiency [28]. In addition, there are works where CDX models of liposarcomas were used to test the effectiveness of doxorubicin and cisplatin to predict therapeutic response in cancer patients [25].

However, the limited number of suitable animal systems, high heterogeneity and low incidence of liposarcomas are the main obstacles to obtaining highly effective *in vivo* models of this disease.

Experimental models of undifferentiated pleomorphic sarcomas

Undifferentiated pleomorphic sarcomas are the most common type and are classified as tumors of indeterminate differentiation, predominantly located in the upper and lower extremities of the body [29]. Surgical intervention leading to disability remains the only radical treatment method for this category of patients due to low incidence of subtypes of undifferentiated pleomorphic sarcoma and lack of large clinical trials. However, disorders of the *TP53, RB1, PTEN, CDKN2A* and *ATRX* genes have been described as associated with the development of this type of sarcoma and can be considered potential therapeutic targets [30–32].

The development of animal models of undifferentiated pleomorphic sarcomas is complicated by problems with the availability and quantity of clinical material [33]. However, in recent decades, primary undifferentiated pleomorphic sarcoma cell

lines and corresponding ectopic mouse models have been obtained [34, 35]. These model systems allowed establishing differences in the effectiveness of doxorubicin *in vivo* and *in vitro*, thereby emphasizing the need to test chemotherapeutic agents not only in cell lines, but also in laboratory animals [34].

Another study showed that models of undifferentiated pleomorphic sarcoma obtained in immunodeficient mice from primary lines were similar in histological characteristics and protein expression of Ki-67 and CD31 to patients' tumor tissues [36]. However, the clonal composition of tumors *in vivo* and patients, as well as their transcriptomic features were different, which caused differences in the effectiveness of doxorubicin, gemcitabine and cisplatin [36].

In addition to the use of tumor cells and primary cell lines, there are examples of subcutaneous implantation of an intact tumor fragment and the formation of viable xenografts that are similar to the original tumors in terms of the content of necrotic cells [37]. Such models are easily reproducible, in contrast to injection of a cell suspension of the primary tumor.

GEM models with pathogenetically significant molecular abnormalities make it possible to evaluate the growth and development of tumors under natural conditions in the body of an experimental animal. For instance, a model of undifferentiated pleomorphic sarcoma using GEM (red fluorescent protein, RFP+) and the introduction of a surgical tumor fragment has become a platform for non-invasive imaging of tumor growth, migration, cell invasion and screening of drug efficacy [38]. A mouse model of undifferentiated pleomorphic sarcoma has also been obtained using the Cre-loxP and CRISPR-Cas9 genome editing systems. The introduction of adenoviral vectors led to the spontaneous formation of tumors similar in histology, morphology and mutational profile [39]. The development of such models is less expensive both time and money-wise compared to traditional GEM models.

Thus, experimental models of undifferentiated pleomorphic sarcoma, mainly GEM, have allowed the development of new therapeutic strategies, including organ-preserving treatments to improve the quality of life of patients.

Experimental models of synovial sarcoma

Synovial sarcoma accounts for 5 % to 10 % of all STS, is characterized by aggressive growth and is associated with the t(X,18) chromosomal translocation encoding the chimeric *SS18-SSX* gene [40, 41]. Although some synovial sarcomas develop near joints, the tumor cells are morphologically dissimilar to the synovium, and the tumor precursor cell remains unknown to date [42]. Surgical resection with or without radiation and/or doxorubicin-based chemotherapy is the mainstay of treatment for patients with synovial sarcoma [43]. The insufficiency of treatment options justifies the relevance of studying the molecular mechanisms of this type of sarcoma and developing new therapeutic solutions.

Major studies of the pathogenesis of synovial sarcoma, like most other tumors, are based on the analysis of cell lines, primary tumor cells and xenografts. A CDX model of synovial sarcoma has been developed using the SW-982 cell line, which is similar to the primary tumor in histological and immunohistochemical terms [44]. There are PDX models of synovial sarcoma, including those used to test the effectiveness of new treatment methods [45–47]. Recently developed GEM models have significantly expanded the scope of preclinical studies of synovial sarcoma (Fig.). Although labor-intensive and expensive, GEM models based on site-specific recombination technology are fundamental tools for understanding the pathogenesis and molecular biology of cancer [48]. Thus, it has been shown that high expression of the chimeric gene *SYT-SSX2* is associated with the development of synovial sarcoma, and myoblasts are potential precursors of tumor cells [49, 50]. In another GEM model, after injection of the TAT-CRE protein (Cre-loxP genetic engineering system), the role of *PTEN* gene alterations in enhancing tumor growth and metastasis of synovial sarcoma was proven [51], which is associated with low patient survival rates [52]. However, despite these advances, the high heterogeneity of synovial sarcoma is the main obstacle to the creation of a universal model system (Fig.).

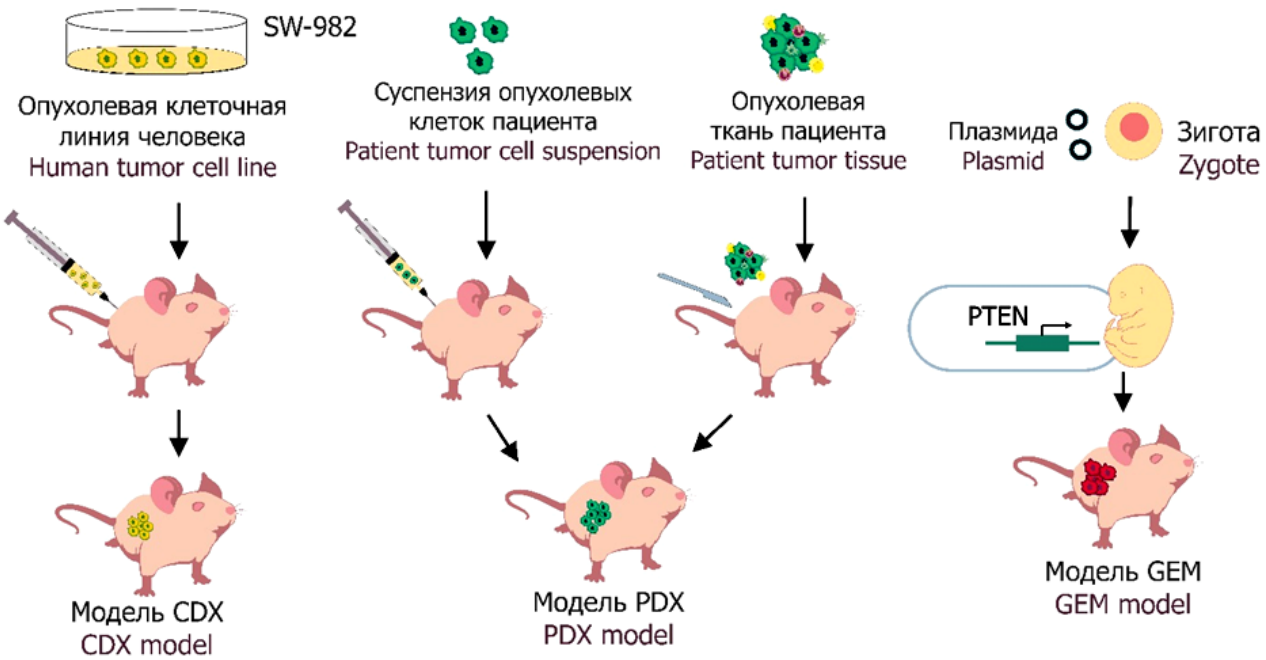

Fig. Different methods for modeling synovial sarcoma *in vivo*

Application of *in vivo* models in therapy

One of the primary objectives of animal models is to screen for the effectiveness of drugs. To date, treatment of various subtypes of STS is based on standard protocols of chemotherapy (doxorubicin, epirubicin, dacarbazine, ifosfamide), a combination of anthracyclines with ifosfamide and/or dacarbazine, and surgical resection [53, 54].

Through *in vivo* models, drug efficacy is screened and new therapeutic approaches are actively developed. Among such approaches, targeted drugs occupy a special place. PDX models of undifferentiated pleomorphic sarcoma allow testing potential agents aimed at different targets, as the xenograft mimics the disease pattern, including invasive growth into surrounding tissues, metastasis, and relapse formation after surgery [55]. The targeted drug temozolomide demonstrated high efficacy in orthotopic PDX models of undifferentiated pleomorphic sarcoma. The combination of gemcitabine/docetaxel and pazopanib was effective in three of the orthotopic PDX models. At the same time, the sensitivity of each subtype of undifferentiated pleomorphic sarcoma to drugs was individual for each of the models. In other words, each subtype of sarcoma was characterized by its own pattern of drug sensitivity [56, 57].

An orthotopic PDX model, derived from a primary tumor in athymic nude mice and mimicking doxorubicin resistance, has also been developed for dedifferentiated liposarcoma. This model shows that metabolic exposure to recombinant methioninase (rMETase) in combination with palbociclib (a CDK4 inhibitor) leads to tumor regression [57]. In addition, in synovial sarcoma, palbociclib inhibits Rb phosphorylation, causing cell cycle arrest in the G1 phase and blocking proliferation. Targeting synovial sarcoma at molecular level is especially relevant, since, unlike most sarcomas that recur and metastasize to lymph nodes, synovial sarcomas are characterized by early distant metastasis to the lungs [58]. For instance, in models of synovial sarcoma, it has been shown that deficiency of INI-1 (integrase

interactor 1) causes the EZH2 protein (enhancer of zeste homolog 2) to acquire oncogenic driver properties and the ability to specifically bind to the chimeric gene *SS18-SSX1* [47]. Thus, targeting EZH2, for example with the drug tazemetostat, is effective in treating patients with synovial sarcoma and other types of cancer [47, 59].

The development of advanced humanized animal models is necessary to study the cellular and molecular factors involved in the immune antitumor response and to develop new immunotherapeutic tools [60]. One of the main directions in immunotherapy is the blockade of immune checkpoints for programmed cell death: PD-1 (programmed cell death protein 1) and PD-L1 (programmed cell death ligand 1) [61]. Dedifferentiated liposarcoma xenografted NOD mice treated with pembrolizumab (anti-PD-1) demonstrated immune cell infiltration and subsequent tumor regression [60]. Pembrolizumab was also effective in clinical trials in the treatment of synovial sarcoma, DDL and undifferentiated pleomorphic sarcoma [62]. Another example of immunotherapy for synovial sarcoma is the use of ipilimumab, which targets the NY-ESO-1 protein (CTAG1B, cancer/testis antigen 1B) [63]. However, the immunological features of the microenvironment of various types of STS remain poorly understood, and further studies, including *in vivo* models, will identify new immunotherapeutic targets.

When discussing the development of new approaches to STS treatment, one cannot ignore physical methods of influencing the tumor. One of them is the ablation method, a non-invasive option based on high-intensity focused ultrasound that allows tissue to be destroyed thermally or mechanically into an acellular homogenate [64]. The safety and effectiveness of one ablative method (histotripsy) for the removal of superficial STS tumors has been demonstrated in large animal models including cats and dogs [64, 65]. Treatment and subsequent tumor resection showed that histotripsy was well tolerated and very effective in dogs with spontaneous STS. It is assumed that histotripsy, in addition to physical destruction, can change the tumor microenvironment, releasing tumor antigens and leading to tumor infiltration by immune cells, thereby causing a local and systemic immune response [65]. Another method is cryoablation, where the pain is reduced through the use of low temperatures [66].

Despite the wide capabilities of *in vivo* models, they have serious disadvantages, including difficulty of obtaining, high cost, and the impossibility of recreating the structure and cellular composition of the original tumor. The latter likely explains the discrepancies in drug efficacy obtained between *in vivo* models and patients. For example, the drug olaratumab (a monoclonal antibody against PDGF receptor alpha) showed antitumor activity in PDX models, but was low effective in clinical trials for metastatic sarcoma [67, 68].

Thus, studies of therapeutic approaches in *in vivo* models expand the possibilities for finding new methods of treating aggressive and heterogeneous forms of STS. Given the low effectiveness of monochemotherapy in cases of inoperable forms or late stages of STS, animal models are a potential tool in predicting the effectiveness of therapy and selecting other treatment methods.

Conclusion

Various animal models are indispensable tools in oncology, allowing for research to identify the molecular and cellular mechanisms of the formation and progression of malignancies, identify targets, develop drugs and therapeutic tools and test their effectiveness. Especially, these models are relevant for STS, characterized by high aggressiveness and heterogeneity, as well as low frequency of occurrence, which complicates the collection of a representative amount of clinical material. Currently, PDX and GEM models are widely used to identify molecules and signaling pathways involved in the development of sarcomas, identify tumor-initiating cells, and assess the chemoresistance of known drugs and new drugs at the level of the entire tumor ecosystem. However, the key problems of STS animal models remain changes in their composition and phenotype compared to the original tumor, poor survival of surgical material, lack of cellular immunity in immunocompetent models, high cost, and the length of time it takes to create and maintain the model. A solution to one of the problems

may be the use of humanized animals with PDX, which implies the presence in the model of immune, stromal and tumor components that are as close as possible to the human body. However, developing such models is an even more expensive and time-consuming task.

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Экспериментальные модели опухолевого роста при саркомах мягких тканей

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Аннотация. Саркомы мягких тканей представляют собой редкие опухоли (около 1 % от всех злокачественных новообразований) и включают более 70 гистологических подтипов, патогенетические особенности которых остаются до конца невыясненными. Во многом это связано как с количеством и объемом клинического материала, так и с высокой гетерогенностью заболевания. Учитывая редкость каждого отдельного подтипа сарком мягких тканей и гетерогенность, остро стоит вопрос о необходимости разработки универсальных модельных систем для понимания молекулярных изменений, определяющих биологию опухоли. К таким системам относят CDX-модели (cell line-derived xenograft), созданные из клеточных линий, PDX (patient-derived xenograft), полученные из клеток первичной опухоли/метастаза как целого фрагмента операционного материала, так и из клеточной суспензии; гуманизированные животные, содержащие различные человеческие иммунные клетки, и GEM (генно-модифицированные модели), которые создаются посредством трансфекции генетических изменений, характерных для различных подтипов сарком мягких тканей. Для создания тест систем используются не только широкодоступные мышиные модели, но и другие животные, такие как рыбы *Danio rerio*, крысы, свиньи и собаки. Другой важной задачей применения животных моделей является скрининг эффективности современных лекарственных препаратов. На сегодняшний день лечение различных подтипов сарком мягких тканей основано на стандартных протоколах химиотерапии (доксорубицин, эпирубицин, дакарбазин, ифосфамид) и хирургической резекции. В случае неоперабельных форм или поздних стадий сарком мягких тканей животные модели являются потенциальным инструментом в предсказании эффективности терапии и персонализированного подбора схем лечения. В этом плане особую актуальность представляют исследования механизмов таргетного воздействия на специфические молекулярные мишени и применение гуманизированных животных для разработки новых подходов иммунотерапии. В данном обзоре обсуждаются животные модельные системы трех наиболее распространенных типов сарком мягких тканей: липосарком, недифференцированных плеоморфных и синовиальных сарком, а также применение данных моделей для поиска новых терапевтических решений. *Выводы*. В настоящее время находят широкое применение PDX и GEM модели, позволяющие идентифицировать молекулы и сигнальные пути, вовлеченные в развитие сарком, выявлять опухоль-инициирующие клетки, оценивать химиорезистентность известных препаратов и новых лекарственных средств на уровне целостной опухолевой экосистемы. Тем не менее, ключевыми проблемами животных моделей саркомы мягких тканей остаются изменение их состава и фенотипа по сравнению с исходной опухолью, плохая приживаемость операционного материала, отсутствие клеточного иммунитета в иммунокомпетентных моделях, дороговизна, длительность создания и поддержания модели. Решением одной из проблем может стать использование гуманизированных животных с PDX, что подразумевает наличие в модели иммунного, стромального и опухолевого компонентов, максимально приближенных к человеческому организму.

Ключевые слова: саркомы мягких тканей, *in vivo* модели, терапия

Информация о финансировании. Работа выполнена при финансовой поддержке Российского научного фонда (проект № 23–65–00003)

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Информация о конфликте интересов. Авторы заявляет об отсутствии конфликта интересов.

Благодарности — не применимо.

Этическое утверждение — не применимо.

Информированное согласие на публикацию — не применимо.

Поступила 16.10.2023. Принята 14.11.2023.

Для цитирования: *Tretyakova M.S., Bokova U.A., Korobeynikova A.A., Denisov E.V.* Experimental models of tumor growth in soft tissue sarcomas // Вестник Российского университета дружбы народов. Серия: Медицина. 2023. Т. 27. № 4. С. 459–469. doi: 10.22363/2313–0245–2023–27–4–459–469

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