*Send Orders for Reprints to reprints@benthamscience.net*

*Current Cancer Drug Targets*, 2023, *23*, 1-6



SHORT COMMUNICATION

# Micro-Fragmented Fat Inhibits the Progression of Human Mesothelioma Xenografts in Mice

Silvia La Monica[1,#], Valentina Coccè[2,#], Mara Bonelli[1,*], Giulio Alessandri[2], Roberta Alfieri[1], Costanza Annamaria Lagrasta[1], Caterina Frati[1], Lisa Flammini[3], Aldo Gianni[1,2], Francesco Petrella[1,2], Francesca Paino[2] and Augusto Pessina[2]

[1]*Department of Medicine and Surgery, University of Parma, Via Gramsci 14, 43126 Parma, Italy;* [2]*Surgical and Dental Sciences, Universita Degli Studi Di Milano, University of Milan, Biomedical, Via della Commenda 10, 20122 Milano, Italy;* [3]*Food and Drug, University of Parma, Via Gramsci 14, 43126 Parma, Italy*

ARTICLE HISTORY

Received: June 23, 2022
Revised: December 12, 2022
Accepted: December 14, 2022

*DOI:*
*10.2174/1568009623666230201092302*

**Abstract:** ***Background*:** Malignant pleural mesothelioma is a pathology with no effective therapy and a poor prognosis. Our previous study demonstrated an *in vitro* inhibitory effect on mesothelioma cell lines of both the lysate and secretome of adipose tissue-derived Mesenchymal Stromal Cells. The inhibitory activity on tumor growth has been demonstrated also *in vivo*: five million Mesenchymal Stromal Cells, injected "*in situ*", produced a significant therapeutic efficacy against MSTO-211H xenograft equivalent to that observed after the systemic administration of paclitaxel.

***Objective*:** The objective of this study is to evaluate the efficacy of less (half a million) Mesenchymal Stromal Cells and micro-fragmented adipose tissues (the biological tissue from which the Mesenchymal Stromal Cells were isolated) on mesothelioma cells growth.

***Methods*:** Tumor cells growth inhibition was evaluated *in vitro* and in a xenograft model of mesothelioma.

***Results*:** The inhibitory effect of micro-fragmented fat from adipose-tissue has been firstly confirmed *in vitro* on MSTO-211H cell growth. Then the efficacy against the growth of mesothelioma xenografts in mice of both micro-fragmented fat and low amount of Mesenchymal Stromal Cells has been evaluated. Our results confirmed that both Mesenchymal Stromal Cells and micro-fragmented fat, injected "*in situ*", did not stimulate mesothelioma cell growth. By contrast, micro-fragmented fat produced a significant inhibition of tumor growth and progression, comparable to that observed by the treatment with paclitaxel. Low amount of Mesenchymal Stromal Cells exerted only a little anticancer activity.

***Conclusion*:** Micro-fragmented fat inhibited mesothelioma cell proliferation *in vitro* and exerted a significant control of the mesothelioma xenograft growth *in vivo*.

**Keywords:** Mesothelioma, micro-fragmented fat, mesenchymal stromal cells, paclitaxel, xenograft, adipose tissue.

## 1. INTRODUCTION

The Malignant Pleural Mesothelioma (MPM) is a tumor related to asbestos exposure that, till now, has no effective therapy and has a poor prognosis [1, 2]. Due to the mesenchymal origin of MPM pathology, it may be important to understand the relationships between cancer and mesenchymal stromal cells (MSCs). In fact, it has been reported that the MSCs can exert both a tumor-promoting function, probably due to their paracrine effects [3], or display antitumor activity, as demonstrated in many different types of cancers [4]. Regarding MPM, a significant tumor-inhibiting effect *in vitro* has been described by conditioned medium (CM) from human lung MSCs [5]. Moreover, our previous studies demonstrated that both lysates and CM from adipose tissue-derived MSCs (AT-MSCs) inhibited mesothelioma cell line proliferation. The *in vivo* study on MPM xenograft mouse model confirmed that a loco-regional treatment with high amounts (five millions) of AT-MSCs resulted in a dramatic inhibition of tumor growth, comparable with that produced by systemic administration of paclitaxel [6]. In the present study we report the *in vivo* effect exerted on MPM xenografts by micro-fragmented fat (MFAT) and "low amount" (half a million) of AT-MSCs. Our results confirmed that both MFAT and MSCs did not stimulate tumor growth, and that MFAT locally injected exerted a remarkable therapeutic efficacy, comparable to that produced by paclitaxel injected intraperitoneally.

*Address correspondence to this author at the Department of Medicine and Surgery, University of Parma, *Via* Gramsci 14, 43126 Parma, Italy; Tel: +39 0521 903766; E-mail: mara.bonelli@unipr.it

[#]*These authors contributed equally to this works.*

1568-0096/23 $65.00+.00 

## 2. MATERIALS AND METHODS

### 2.1. Tumor Cell Lines

The human mesothelioma cell line MSTO-211H [7] was obtained from ATCC (Manassas, VA, USA), which authenticates the phenotype of the cell line on a regular basis. Cells were cultured in RPMI-1640 supplemented with 10% Fetal Bovine Serum (FBS, Euroclone, Milan, Italy) and maintained at 37° C in a water-saturated atmosphere of 5% $CO_2$ in air. The cell line was routinely tested for mycoplasma contamination.

### 2.2. Micro-fragmented Adipose Tissue and MSCs

Adipose tissue lipoaspirates from adult donors were collected after written informed consent in accordance with the Declaration of Helsinki. The approval of the collection was obtained from the Institutional Ethical Committee of IRCCS Cà Granda Ospedale Maggiore Policlinico of Milan (#978) and concerned redundant biological material to use for the research that was otherwise discarded. MFAT was prepared as previously described [8] starting from lipoaspirates that were micro-fragmented using an appropriate device (Lipogems® International, Milan, Italy). The final MFAT was stored at -20°C until use. From the same lipoaspirate, the MSCs were obtained as previously described [9]. Briefly, lipoaspirate was treated by enzymatic digestion with type I collagenase (Life technologies, USA) and after centrifugation (1000xg, 15 min), the floating fraction was discarded, and cellular pellet was collected. Cellular pellet was plated on 25$cm^2$ flask (Euroclone, Italy) and expanded in DMEM low glucose medium (Euroclone, Milan, Italy) supplemented with 5% platelet lysate Stemulate (Cook Reagent, USA) and 2 mM L-glutamine (Euroclone, Italy) by incubation at 37°C, 5% $CO_2$. Primary cultures were analyzed for their proliferation rate (Population doubling time), clonogenicity (CFU-F assay), the expression of the typical mesenchymal stem cell markers and multi-differentiate ability towards mesodermal lineages (osteogenic, adipogenic and chondrogenic differentiation) as previous described [6].

### 2.3. Analysis of Cell Proliferation

The effect of MFAT on MSTO-211H cell proliferation was analyzed using transwell inserts. MSTO-211H ($6x10^3$ cells) were seeded in a 24-well plate (diameter 1.9 $cm^2$), while aliquots of MFAT (50, 100, 200 µl) were seeded into the insert (5 µm pore size; Costar, Corning). After 4 days of incubation (37°C, 5% $CO^2$), the inserts were removed and the cells in the well were stained with 0.25% crystal violet (Sigma Aldrich, St Louis, MO) for 10 min, washed with PBS buffer and eluted with 0.3 ml of 33% glacial acetic acid. The absorbance of the eluted dye was measured at 550 nm. Aliquots of PBS 1x (50, 100, 200 µl) were used in inserts as negative controls.

### 2.4. *In Vivo* Experiments

The study was performed in a subcutaneous mouse xenograft model as previously described (6). Briefly Balb/c-Nude female mice (Charles River Laboratories, Calco, Italy) were subcutaneously injected with $10^6$ human MPM MSTO-211H cells suspended in 200 µl of Matrigel (BD Biosciences, Erembodegem, Belgium)/PBS (1:1). The mesothelioma bearing mice were then randomized in 4 groups: control (CTRL, n=4), paclitaxel (PTX, n=5), mesenchymal cells (MSC, n=5) and microfragmented fat (MFAT, n=6). Once a week, vehicle alone (control group) or paclitaxel (20mg/kg) was administered intraperitoneally and a total of 5 x $10^5$ of MSCs in 200 µL of Matrigel and PBS (1:1) or 200µl of MFAT were subcutaneously injected very close to the tumor. After three treatments at days 0, 7, 14, the PTX, MFAT and MSCs administration was suspended for further 6 days to evaluate tumor mass growth after the last treatment. Tumor xenografts were measured twice a week using a digital caliper and tumor volume was determined using the formula: (length x $width^2$)/2 as previously described [10]. At day 20, mice were euthanized and the tumor nodules were collected for further analyses. All experiments involving animals and their care were performed with the approval of the Local Ethical Committee of the University of Parma (Organismo per la Protezione e il Benessere degli Animali, OPBA) and the Italian Ministry of Health, in accordance with the institutional guidelines that are in compliance with national (D. Lgs. 26/2014) and international (Directive 2010/63/EU) laws and policies. As previously described [11], the tumor masses, excised, formalin fixed and paraffin embedded were processed for histochemical analysis. Briefly, the morphometric evaluation of xenograft composition was performed on Masson's trichrome-stained sections to evaluate neoplastic, fibrotic and necrotic tissue. These evaluations were performed on the entire section of each tumor sample using an optical microscope (200X final magnification).

### 2.5. Statistical Analysis

Data are expressed as the mean ± standard error (SE). For statistics, one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test (to adjust for pairs comparisons) was performed by using GraphPadINSTAT program (GraphPad Software Inc., San Diego, CA, USA). p values $< 0.05$ were considered statistically significant.

## 3. RESULTS

### 3.1. *In Vitro* Effects of MFAT on Proliferation of MSTO-211H Cells

As first we evaluated the MFAT effect on MPM cell growth. As shown in (Fig. **1**), the exposure of MSTO-211H cells to MFAT produced inhibition of cell proliferation, as shown by the percent of growth inhibition versus untreated control cells. The inhibition of cell proliferation was dose dependent: 50 ul of MFAT did not affect the cell growth, 100 ul produced a 30 % of inhibition (even if it was not statistically significant), and 200 µl of MFAT produced a 70 % significant ($p<0.05$) inhibition of MSTO-211H proliferation. On the contrary, exposure to the same volumes of PBS did not produce any significant effect on cell growth (as shown in Fig. **1A**).

### 3.2. *In Vivo* Effects of MFAT and MSCs in a MSTO-211H Xenograft Model

We then investigated the efficacy against the growth of mesothelioma xenografts in mice of both MFAT and low

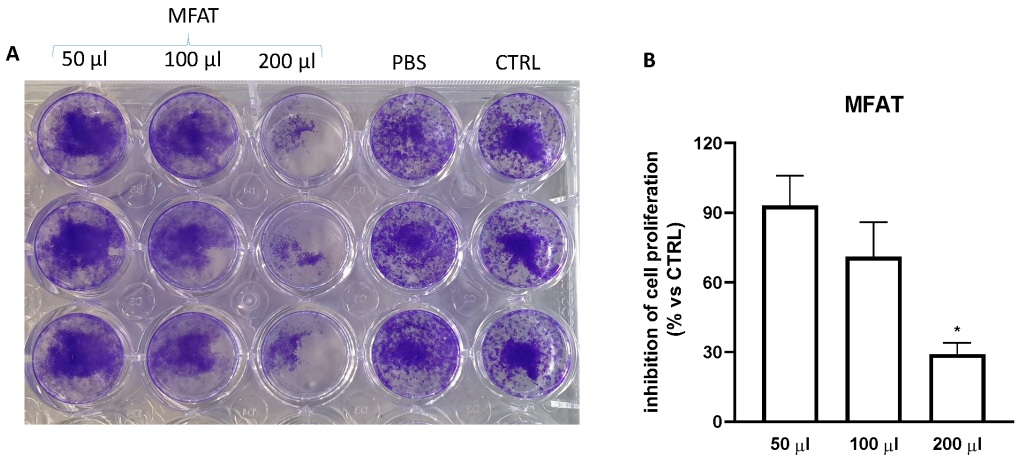


**Fig. (1).** Inhibitory effect of MFAT on proliferation in mesothelioma cells. **A**) Representative image of 24 wells plate with MSTO-211H cells stained with crystal violet after 4 days of PBS or MFAT exposition (50, 100, 200 µl) in transwell inserts (pore size 5µm). **B**) The histogram reports the MSTO-211H proliferation expressed as % vs CTRL after crystal violet elution with acetic acid (mean ± standard error (SE) of 15 replicates) (*p<0.05). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

amount (half a million) of MSCs isolated by the same donor of lipoaspirate. The effect on neoplastic growth, expressed as volume of growing tumor mass (Fig. **2**) clearly indicates that the *in situ* treatment with MFAT (at days 0, 7, 14) produced the same inhibition pattern of the neoplastic progression as observed by treating mice with PTX intraperitoneally at 10 mg/kg. At day 14 when the mice received the third treatment, a significant (p<0.05) lower volume of tumor was detected with PTX and MFAT. On the contrary, we did not observe a significant effect on tumor volume reduction with low doses of MSCs, differently from our previous findings using large amounts (5 x 106) of MSC. Interestingly, after 3 and 6 days from the last treatment, tumor volume did not show a significant increase in mice treated with MFAT, whereas we observed a reduction in tumor progression in mice treated with PTX or MSCs, even if not statistically significative (Fig. **3A**). The greater reduction in tumor volume in mice treated with MFAT was highlighted more clearly by plotting the difference between the tumor volume mean at day 20 and 14 (Fig. **3B**). It is worth noting that the tumors of mice treated with MFAT presented an abundant adipose component (as shown in Fig. **3C**), indicating that the measure of these tumor volume might be overestimated. The morphometric evaluation of xenograft composition did not show significant differences among groups. Indeed, the neoplastic, fibrotic and necrotic tissue fraction was not influenced by the treatment performed for each experimental group (Fig. **4**).

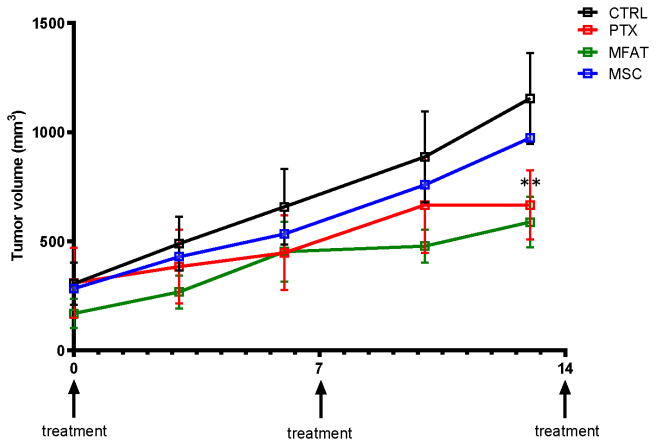


**Fig. (2).** Effects of loco-regional treatment of MSTO-211H xenograft with MSCs and MFAT compared to PTX. The graph represents the tumor volume expressed in $mm^3$ (y axis) after treatment at days 0, 7, 14 (arrows, x-axis), versus untreated control (CTRL) animals. Values are expressed as mean ± standard error (SE); **p<0.01. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

## 4. DISCUSSION

Our preliminary *in vitro* study evidenced a clear trend of inhibition of MPM cell proliferation exerted by increasing amounts of MFAT. Based on this observation we verified if this effect was also exerted on the growth of this mesothelioma cell line in mice xenografts. As expected, the *in vivo* experiments demonstrated that the different treatments did not completely block the tumor growth progression. In particular, tumor volume increases were also observed after the treatment with PTX, confirming the high aggressiveness of this type of tumor. Our present study shows that even very low amounts of adipose derived MSCs ($5x10^5$ cells, corresponding to $5x10^7$ MSC/kg) exert, although poor, a little inhibition of tumor progression. This is in agreement with our previous report demonstrating that ten times higher amounts of MSCs ($5x10^6$ cells, corresponding to $5x10^8$ MSCs/kg) were effective in producing a significant inhibition of mesothelioma growth [6] and very importantly, this confirmed that MSCs do not exert any stimulation on mesothelioma growth. A more interesting observation was that MFAT alone (the biological tissue from which the MSCs were isolated) produced "per se" an effect on the tumor growth equivalent to that exerted by PTX administered systematically. Moreover, after the last treatment, the tumor progression in mice treated with MFAT was slower than that found in mice treated with PTX. Although it is necessary to better understand the mechanism to explain this effect, our

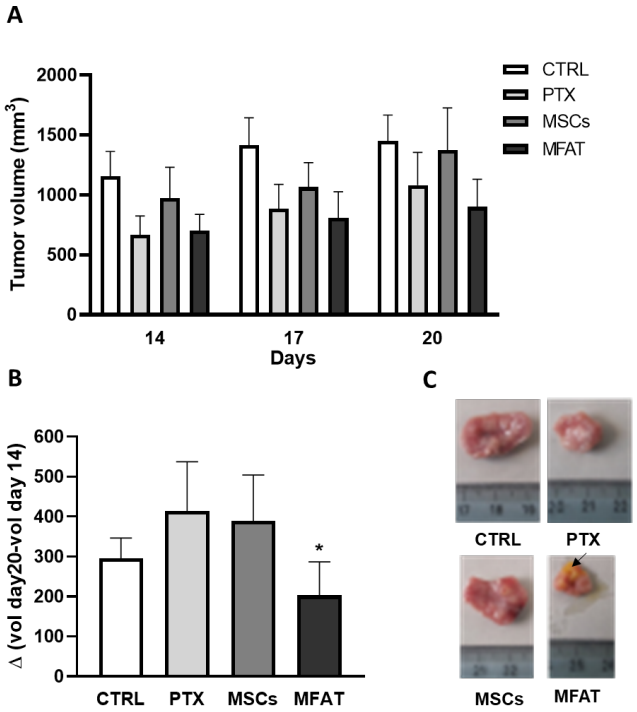


**Fig. (3). A**) MSTO-211H xenograft growth at days 14, 17 and 20 corresponding to 3 and 6 days after the last treatment. The histogram represents the tumor volume expressed in $mm^3$ (y axis) on day 14, 17, 20, compared to the untreated control animals. **B**) Increased tumor volume 6 days after the last treatment. The increment is expressed as a difference (Δ) between the tumor volumes at day 20 and 14 day. Values are expressed as mean ± standard error (SE); *p <0.05. C: Representative images of tumors. Black arrow indicates the adipose component. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

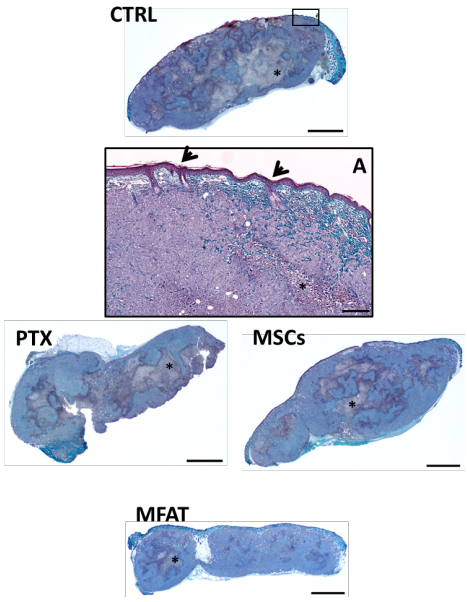


**Fig. (4).** Macrophotographs of Masson's Trichrome stained sections of subcutaneous MPM. MSTO-211H-induced tumor nodules from untreated (CTRL), PTX-treated, MSCs- and MFAT- injected animals. Scale bars = 2 mm. At higher magnification **A**) collagen deposition (greenish) between neoplastic cells (purple) is apparent. Black asterisks indicate necrotic areas and black arrows point to adnexa and skin. Scale bar = 500 µm. (CTRL) animals. Values are expressed as mean ± standard error (SE); **p<0.01. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

observation is in agreement with data reported by other authors indicating the important biological role of both adipocytes and lipid droplets in controlling cancer progression [12, 13]. In particular, some reports pointed out that fatty acid, sphingolipids, ceramides and diglycerols (of which MFAT is rich) have different proven anticancer activities [14-18]. Furthermore, adipocyte, lipid droplets and some fatty acids may act by increasing tissue selectivity to the drugs, making chemotherapy potentially more effective and less toxic [19]. These observations suggest that MFAT could be considered a biocompatible vehicle to deliver anticancer drugs [8, 20, 21]. Of course, our results open some queries that need to be answered by further studies but at the same time they allow to reach some preliminary interesting conclusions: as first, both adipose tissue and MSCs isolated from that do not promote the growth of subcutaneous xenografts of mesothelioma; second, MFAT "per se" inhibit the mesothelioma proliferation *in vitro* and can exert a significant control of the mesothelioma xenograft growth in mice.

## CONCLUSION

Micro-fragmented adipose tissue obtained by lipoaspirate exerted anti-cancer activity against malignant pleural mesothelioma cells, both *in vitro* in and *in vivo* in a mouse xenograft model.

## LIST OF ABBREVIATIONS

MFAT = Micro-Fragmented Fat

MPM = Malignant Pleural Mesothelioma

MSCs = Mesenchymal Stromal Cells

CM = Conditioned Medium

AT-MSCs = Adipose Tissue-derived Mesenchymal Stromal Cells

PTX = Paclitaxel

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experiments involving animals and their care were performed with the approval of the Local Ethical Committee of University of Parma (Organismo per la Protezione e il Benessere degli Animali, OPBA) and of the Italian Ministry of Health.

## HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All the reported experiments were in accordance with the institutional guidelines that are in compliance with national (D. Lgs. 26/2014) and international (Directive 2010/63/EU) laws and policies.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on request from the corresponding author.

## FUNDING

This work was partially supported by the IRCCS European Institute Foundation and by the Italian Ministry of Health with Ricerca Corrente and "5 X 1000" funds.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Declared none.

## REFERENCES

[1] Carbone, M.; Ly, B.H.; Dodson, R.F.; Pagano, I.; Morris, P.T.; Dogan, U.A.; Gazdar, A.F.; Pass, H.I.; Yang, H. Malignant mesothelioma: facts, myths, and hypotheses. *J. Cell. Physiol.,* **2012**, *227*(1), 44-58. http://dx.doi.org/10.1002/jcp.22724 PMID: 21412769

[2] Mutti, L.; Peikert, T.; Robinson, B.W.S.; Scherpereel, A.; Tsao, A.S.; de Perrot, M.; Woodard, G.A.; Jablons, D.M.; Wiens, J.; Hirsch, F.R.; Yang, H.; Carbone, M.; Thomas, A.; Hassan, R. Scientific Advances and New Frontiers in Mesothelioma Therapeutics. *J. Thorac. Oncol.,* **2018**, *13*(9), 1269-1283. http://dx.doi.org/10.1016/j.jtho.2018.06.011 PMID: 29966799

[3] Li, G.C.; Zhang, H.W.; Zhao, Q.C.; Sun, L.; Yang, J.J.; Hong, L.; Feng, F.; Cai, L. Mesenchymal stem cells promote tumor angiogenesis *via* the action of transforming growth factor β1. *Oncol. Lett.,* **2016**, *11*(2), 1089-1094. http://dx.doi.org/10.3892/ol.2015.3997 PMID: 26893697

[4] Ayuzawa, R.; Doi, C.; Rachakatla, R.S.; Pyle, M.M.; Maurya, D.K.; Troyer, D.; Tamura, M. Naïve human umbilical cord matrix derived stem cells significantly attenuate growth of human breast cancer cells *in vitro* and *in vivo*. *Cancer Lett.,* **2009**, *280*(1), 31-37. http://dx.doi.org/10.1016/j.canlet.2009.02.011 PMID: 19285791

[5] Cortes-Dericks, L.; Froment, L.; Kocher, G.; Schmid, R.A. Human lung-derived mesenchymal stem cell-conditioned medium exerts *in vitro* antitumor effects in malignant pleural mesothelioma cell lines. *Stem Cell Res. Ther.,* **2016**, *7*(1), 25. http://dx.doi.org/10.1186/s13287-016-0282-7 PMID: 26861734

[6] Coccè, V.; La Monica, S.; Bonelli, M.; Alessandri, G.; Alfieri, R.; Lagrasta, C.A.; Madeddu, D.; Frati, C.; Flammini, L.; Lisini, D.; Marcianti, A.; Parati, E.; Paino, F.; Giannì, A.; Farronato, G.; Falco, A.; Spaggiari, L.; Petrella, F.; Pessina, A. Inhibition of human malignant pleural mesothelioma growth by mesenchymal stromal Cells. *Cells,* **2021**, *10*(6), 1427. http://dx.doi.org/10.3390/cells10061427 PMID: 34201002

[7] Bepler, G.; Koehler, A.; Kiefer, P.; Havemann, K.; Beisenherz, K.; Jaques, G.; Gropp, C.; Haeder, M. Characterization of the state of differentiation of six newly established human non-small-cell lung cancer cell lines. *Differentiation,* **1988**, *37*(2), 158-171. http://dx.doi.org/10.1111/j.1432-0436.1988.tb00806.x PMID: 2840315

[8] Alessandri, G.; Coccè, V.; Pastorino, F.; Paroni, R.; Dei Cas, M.; Restelli, F.; Pollo, B.; Gatti, L.; Tremolada, C.; Berenzi, A.; Parati, E.; Brini, A.T.; Bondiolotti, G.; Ponzoni, M.; Pessina, A. Microfragmented human fat tissue is a natural scaffold for drug delivery: Potential application in cancer chemotherapy. *J. Control. Release,* **2019**, *302*, 2-18. http://dx.doi.org/10.1016/j.jconrel.2019.03.016 PMID: 30890444

[9] Ceserani, V.; Ferri, A.; Berenzi, A.; Benetti, A.; Ciusani, E.; Pascucci, L.; Bazzucchi, C.; Coccè, V.; Bonomi, A.; Pessina, A.; Ghezzi, E.; Zeira, O.; Ceccarelli, P.; Versari, S.; Tremolada, C.; Alessandri, G. Angiogenic and anti-inflammatory properties of micro-fragmented fat tissue and its derived mesenchymal stromal cells. *Vasc. Cell,* **2016**, *8*(1), 3. http://dx.doi.org/10.1186/s13221-016-0037-3 PMID: 27547374

[10] La Monica, S.; Cretella, D.; Bonelli, M.; Fumarola, C.; Cavazzoni, A.; Digiacomo, G.; Flammini, L.; Barocelli, E.; Minari, R.; Naldi, N.; Petronini, P.G.; Tiseo, M.; Alfieri, R. Trastuzumab emtansine delays and overcomes resistance to the third-generation EGFR-TKI osimertinib in NSCLC EGFR mutated cell lines. *J. Exp. Clin. Cancer Res.,* **2017**, *36*(1), 174. http://dx.doi.org/10.1186/s13046-017-0653-7 PMID: 29202823

[11] La Monica, S.; Minari, R.; Cretella, D.; Flammini, L.; Fumarola, C.; Bonelli, M.; Cavazzoni, A.; Digiacomo, G.; Galetti, M.; Madeddu, D.; Falco, A.; Lagrasta, C.A.; Squadrilli, A.; Barocelli, E.; Romanel, A.; Quaini, F.; Petronini, P.G.; Tiseo, M.; Alfieri, R. Third generation EGFR inhibitor osimertinib combined with pemetrexed or cisplatin exerts long-lasting anti-tumor effect in EGFR-mutated pre-clinical models of NSCLC. *J. Exp. Clin. Cancer Res.,* **2019**, *38*(1), 222. http://dx.doi.org/10.1186/s13046-019-1240-x PMID: 31138260

[12] Antunes, P.; Cruz, A.; Barbosa, J.; Bonifácio, V.D.B.; Pinto, S.N. Lipid Droplets in Cancer: From Composition and Role to Imaging and Therapeutics. *Molecules,* **2022**, *27*(3), 991. http://dx.doi.org/10.3390/molecules27030991 PMID: 35164256

[13] Cruz, A.L.S.; Barreto, E.A.; Fazolini, N.P.B.; Viola, J.P.B.; Bozza, P.T. Lipid droplets: platforms with multiple functions in cancer hallmarks. *Cell Death Dis.,* **2020**, *11*(2), 105. http://dx.doi.org/10.1038/s41419-020-2297-3 PMID: 32029741

[14] Abedin, M.R.; Barua, S. Isolation and purification of glycoglycerolipids to induce apoptosis in breast cancer cells. *Sci. Rep.,* **2021**, *11*(1), 1298. http://dx.doi.org/10.1038/s41598-020-80484-x PMID: 33446783

[15] Zhai, L.; Sun, N.; Han, Z.; Jin, H.; Zhang, B. Liposomal short-chain C6 ceramide induces potent anti-osteosarcoma activity *in vitro* and *in vivo*. *Biochem. Biophys. Res. Commun.,* **2015**, *468*(1-2), 274-280. http://dx.doi.org/10.1016/j.bbrc.2015.10.113 PMID: 26505795

[16] Choi, M.K.; Song, I.S. Recent advances in the formulation of sphingolipid anticancer therapeutics. *J. Pharm. Investig.,* **2020**, *50*(3), 295-307. http://dx.doi.org/10.1007/s40005-020-00475-y

[17] Lin, I.L.; Chou, H.L.; Lee, J.C.; Chen, F.W.; Fong, Y.; Chang, W.C.; Huang, H.W.; Wu, C.Y.; Chang, W.T.; Wang, H.M.D.;

Chiu, C.C. The antiproliferative effect of C2-ceramide on lung cancer cells through apoptosis by inhibiting Akt and NFκB. *Cancer Cell Int.,* **2014**, *14*(1), 1. http://dx.doi.org/10.1186/1475-2867-14-1 PMID: 24393431

[18] Huang, W.C.; Chen, C.L.; Lin, Y.S.; Lin, C.F. Apoptotic sphingolipid ceramide in cancer therapy. *J. Lipids,* **2011**, *2011*, 565316. http://dx.doi.org/10.1155/2011/565316 PMID: 21490804

[19] Jóźwiak, M.; Filipowska, A.; Fiorino, F.; Struga, M. Anticancer activities of fatty acids and their heterocyclic derivatives. *Eur. J. Pharmacol.,* **2020**, *871*, 172937. http://dx.doi.org/10.1016/j.ejphar.2020.172937 PMID: 31958454

[20] Liang, T.; Wen, D.; Chen, G.; Chan, A.; Chen, Z.; Li, H.; Wang, Z.; Han, X.; Jiang, L.; Zhu, J.J.; Gu, Z. Adipocyte-derived anticancer lipid droplets. *Adv. Mater.,* **2021**, *33*(26), 2100629. http://dx.doi.org/10.1002/adma.202100629 PMID: 33987883

[21] Wen, D.; Wang, J. Adipocytes as anticancer. *Matter,* **2019**, *1*(5), 1203-1214.