Anti-tumor activity of B0052 and a novel Korean medicine, in murine melanoma models and relations of composition changes of primo system.

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2010.09.11.

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Contents

I.Introduction : Malignant Melanoma

- Rapid growing tumor without effective therapies.
- Early detection ,surgical removal is the only choice for long term survival
- Inhibition and control of metastasis is important issue.
- Angiogenesis, inflammatory reaction,immune response are important factor
- Primo-vascular system may acts as an additioinal route for cancer migration

Clinical experience

- **Recurred over and over, had resection more than 8times.**
- **Treated with MSB0052 3 month.**
- **Had partial response .**

2009.7 MR

2009.6.9

2009.8.18

II.Preliminary study

- Duration;(2010.4.15 - 2010.5.6) for 20days
- Cell line : B16F10 mouse melanoma cell
- Female nude mice: **(BALB-C-nu/nu)** 6weeks
- cultivated in DMEM
- Injected into the skin on the back
- Grouping

A - control group

- n = 3
- melanoma cell injection
- treated with water

B - drug treated group

n =3

melanoma cell injection

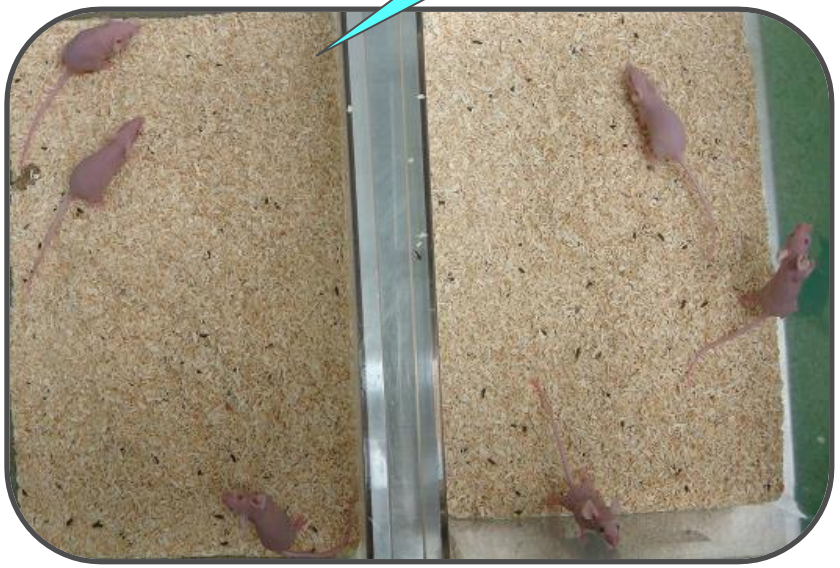
treated with MSB0052 from the same day

Melanoma cancer cell line and in vivo treatment

Inocculation
Into the skin
On the back

Treatment start: 2010.04.15

Divide into 2 group



Melanoma cells were then resuspended in 1mL of DMEM and injected of nude mouse mice for the development of skin cancer.

2010.04.26 10days after

The treatment was started 10 days after tumor cell inoculation.

Control group

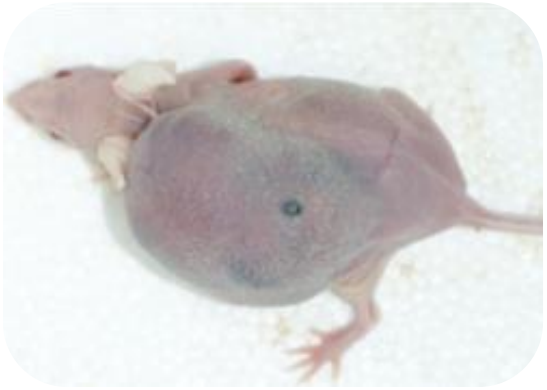
MSB0052 group

Animals (**n = 3 animals** per group) were treated with inhalation of **MSB0052** or **vehicle** once a day for 14 days, or were part of the control group that was not subjected to any treatment.

2010.05.06 after 20days

Control group

MSB0052 group



Necrosis
Melanization
is apparent

Design

- Based on the result shown on preliminary study
- We designed 2nd study

III. Materials and Methods

Cell culture and reagents

- **B16F10 mouse melanoma cell** lines were purchased
  Cells were cultured in DMEM medium

Animal model

- female nude mice **(BALB-C-nu/nu)**
  aged 6 weeks old, 17-20g, n=34

Cell count (**2.5×10^6 cells per animals**)

Cell injection

- Anaesthetized by inhalation of 2% isoflurance in 100% oxygen at a flow rate of 2L min^{-1}
  under the skin into nude mice on the back (腎俞穴)

Study design

- **Divided into three group**

Evaluation of tarket and Histological analysis

- **Weight**
- **Tumor size**
- **Survival**
- **Behavior & activity**
- **Microscopic pathology**
  - Stained with heamtoxylin and eosin(H&E)
  - Organs- axillary,lumbar, inguinal lymph nodes, lung
  - Collect 5days(n=1) 10days (n=1), 15 days(n=3)/each group
  - made into 5 μm sections
- **primo-vascular study**
  - difference of composition between 2 group

Divide 3 group

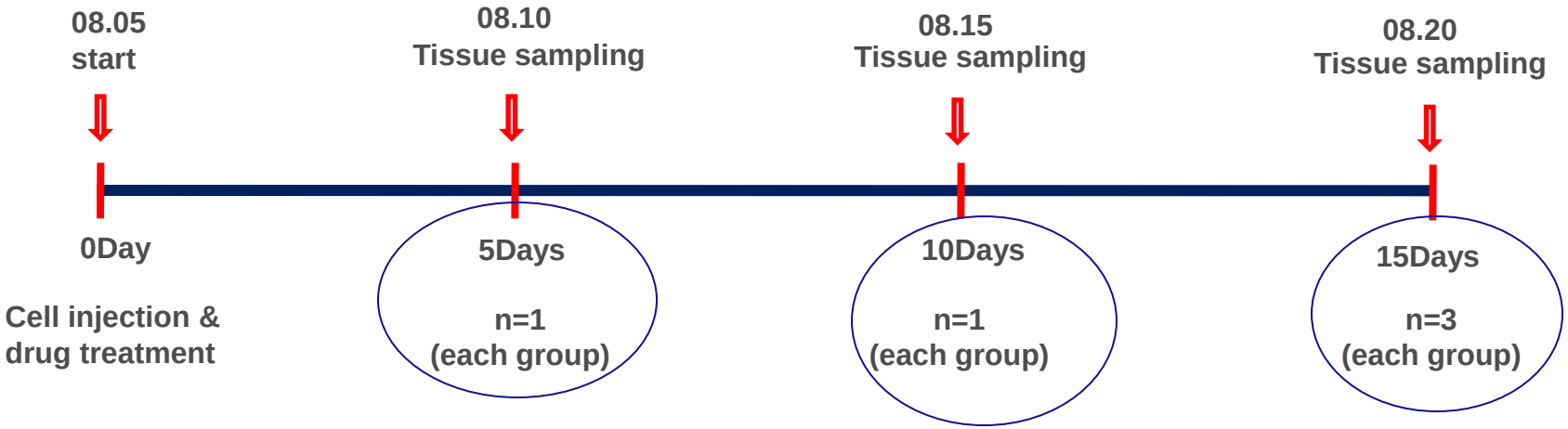
mice were randomly divided into 3 groups

(a) native group	(b) control group	(c) drug Tx group
•Non injected mice	• Melanoma cell injected mice	• Melanoma cell injected mice
•**treated with water** (1ml/20g)	• **treated with water** (1ml/20g)	• **treated with MSB0052** (30mgdrug/1mL/20g)
•Every day three times • n= 4 / for weighting	• Every day three times • n = 15 • - 5 survivol • - 5 histologic purpose • - 5 primo-vascular	• every day three times • n = 15 • -5 survival • -5 hisologic purpose • -5 primo-vascular

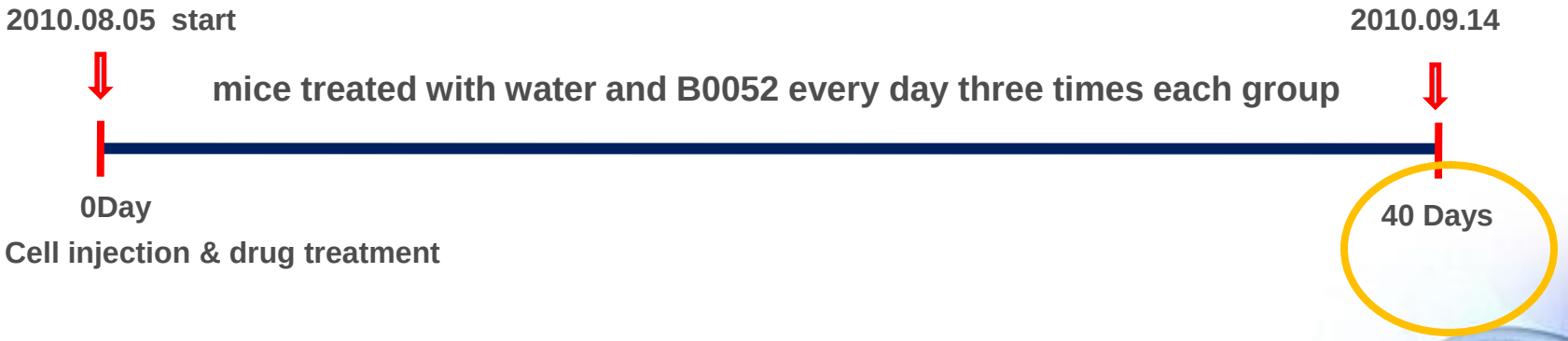
Animal model and study design

Two sets of experimental models were used:

First, Histological analysis



Second, Survival analysis



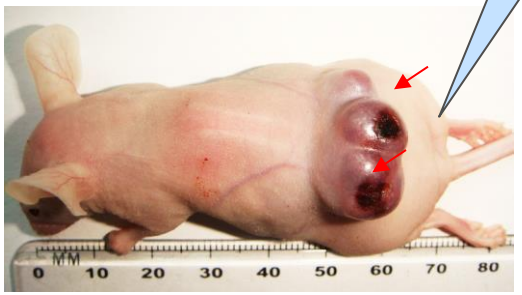
IV. Results

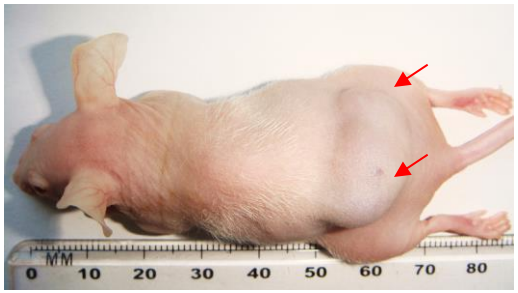
Gross findings of melanoma masses after 15 days

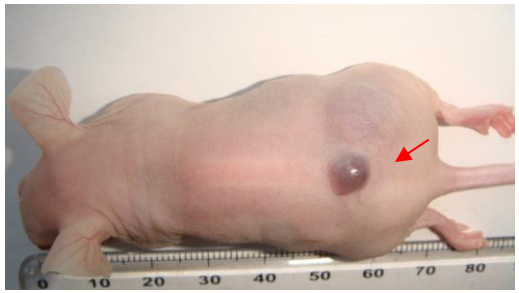
Control group
With water

Drug tx group
MSB0052

larger
& **darker**
Necrosis
was apparent compare with drug treated group





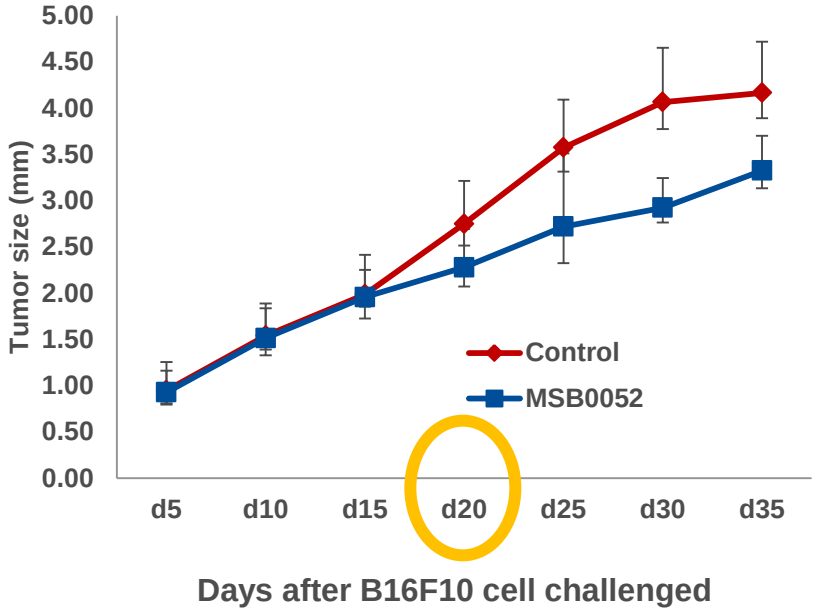


Comparison of the tumor volume after 15day.

MSB0052 inhibited tumor growth in vivo

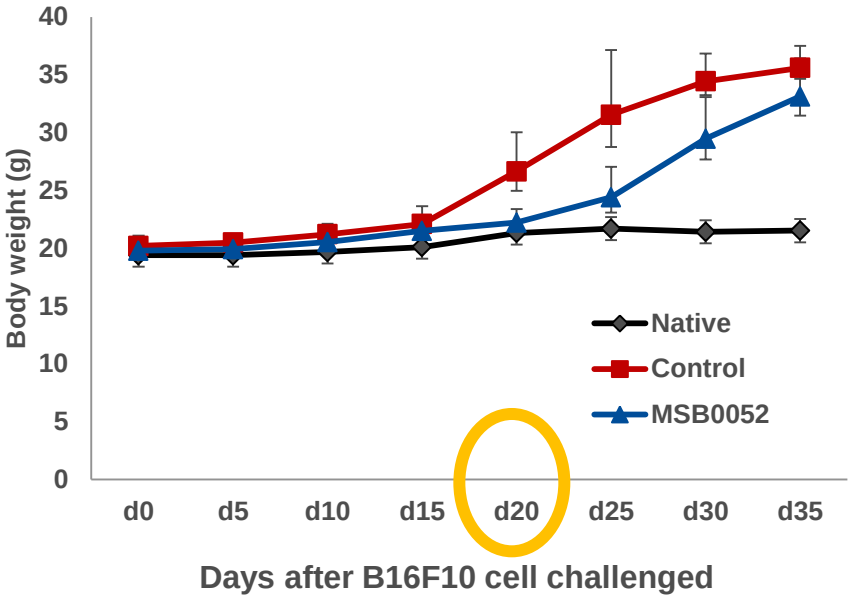
Tumor size

(a)



Body weight

(b)



Anti-tumor effect of MSB0052 in vivo..

(a) Tumor size growth curve. Tumor sizes were measured every 5 days.

There was a difference between MSB0052 group and control groups

(b) Body weight curve. There was a difference in body weight between MSB0052 and control groups

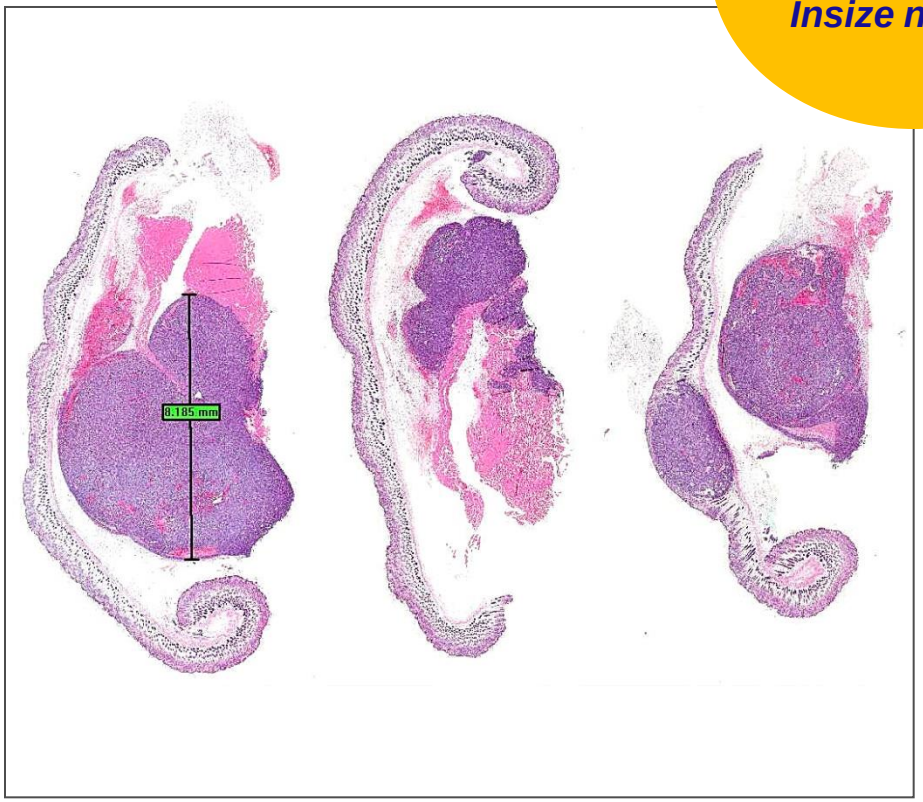
MSB0052 difference body weight and tumor growth after 20 days.

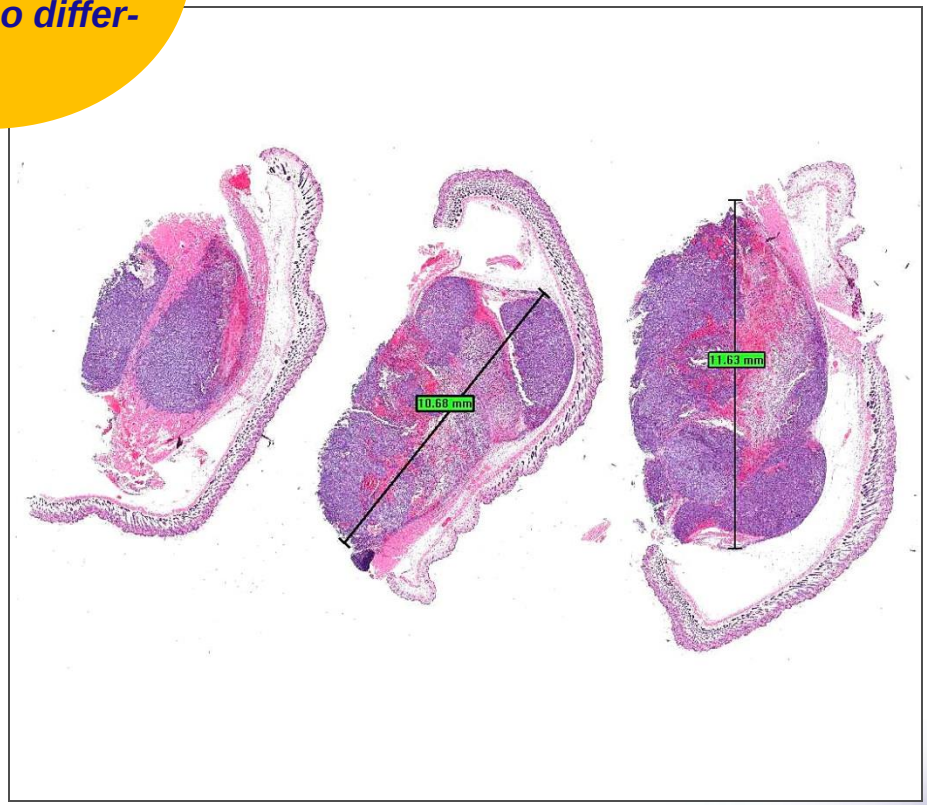
Pathological comparison after 10 days

Control group
With water n =1

After 10 days
Insize no differ-

Drug tx group
With MSB0052 n = 1



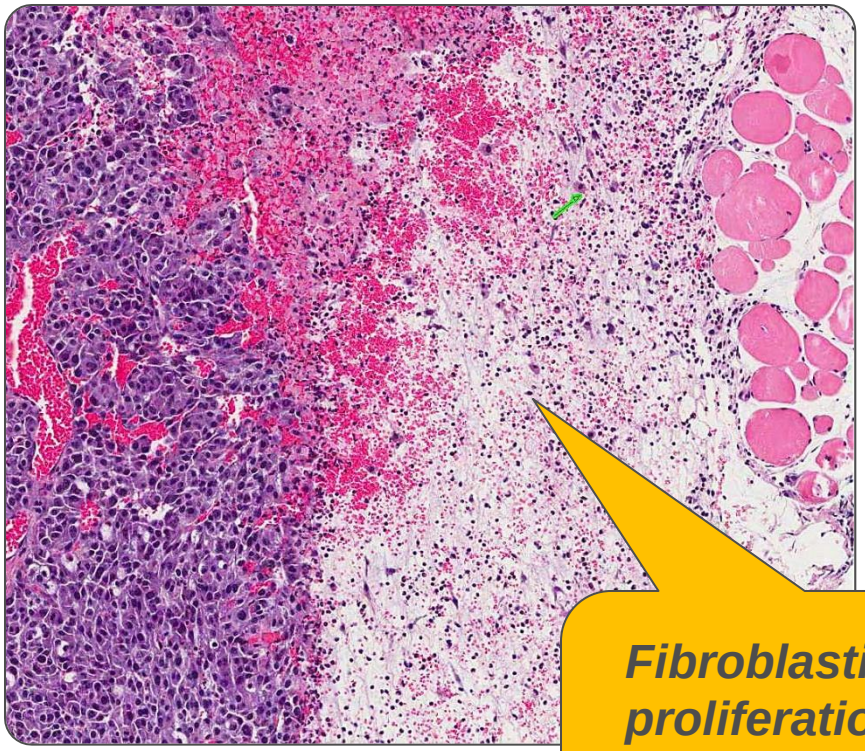


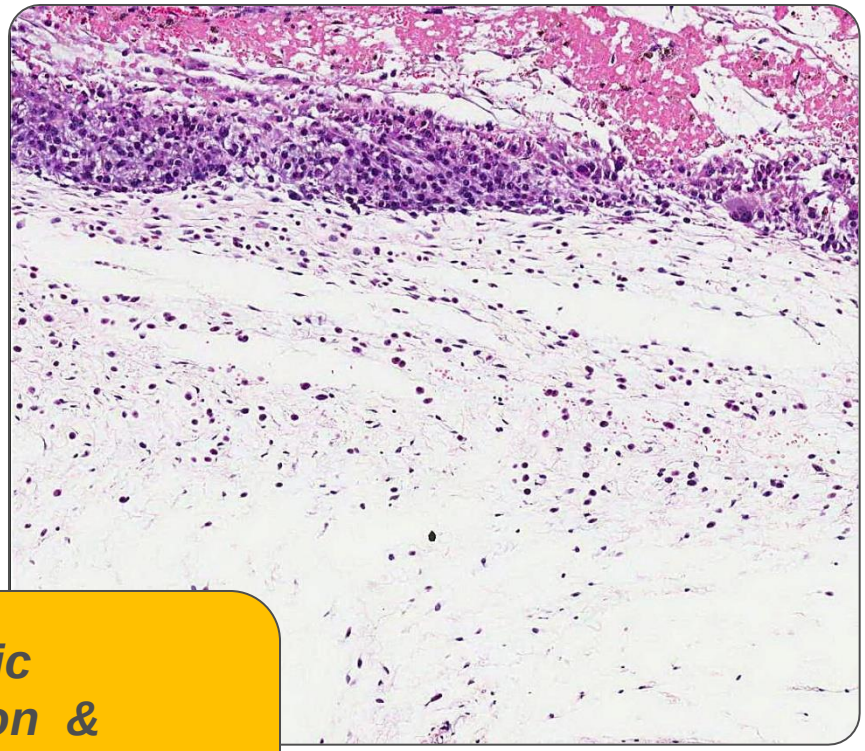
Tumor volume growth. There was a difference in tumor volume between MSB0052 and control groups .

Micro in Control and MSB0052-10 D: Peritumoral reaction I

Control group
With water

Drug tx group
MSB0052

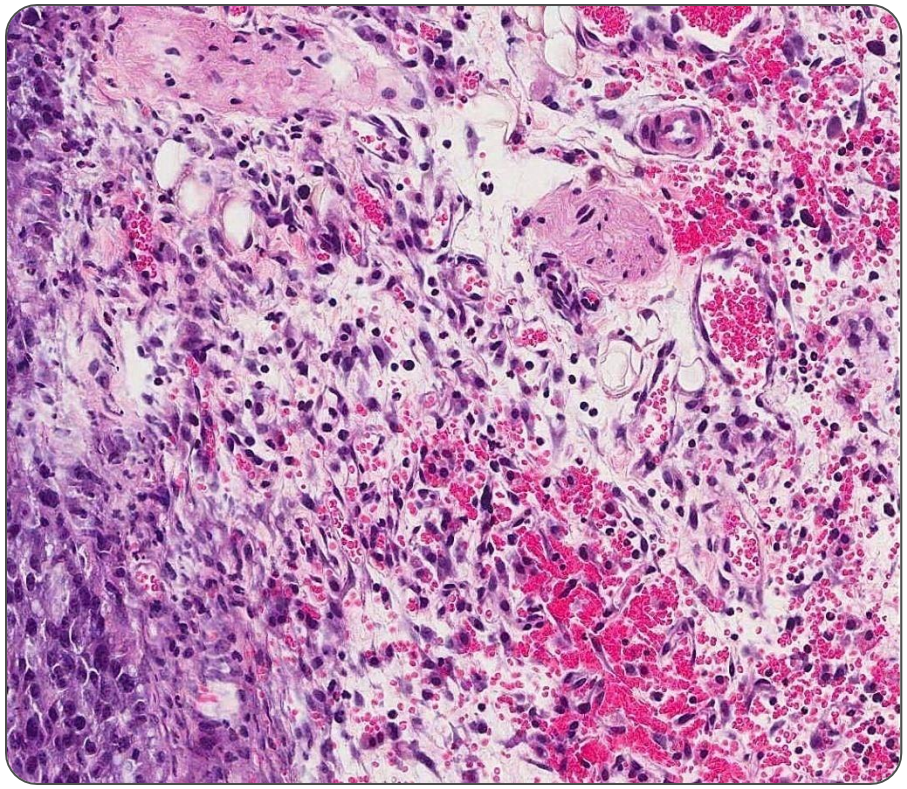




Fibroblastic proliferation & Angiogenesis is apparent

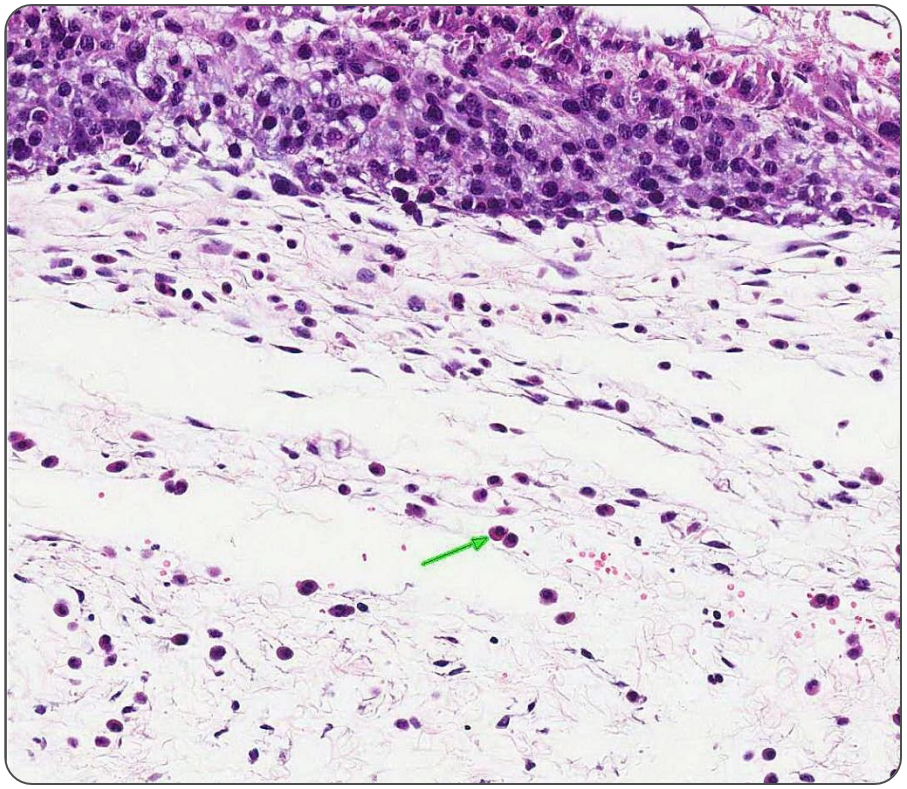
Micro in Control and MSB0052-10 D: Peritumoral reaction I

Control group
With water



Fibroblasts, angiogenesis, neutrophil is prominent

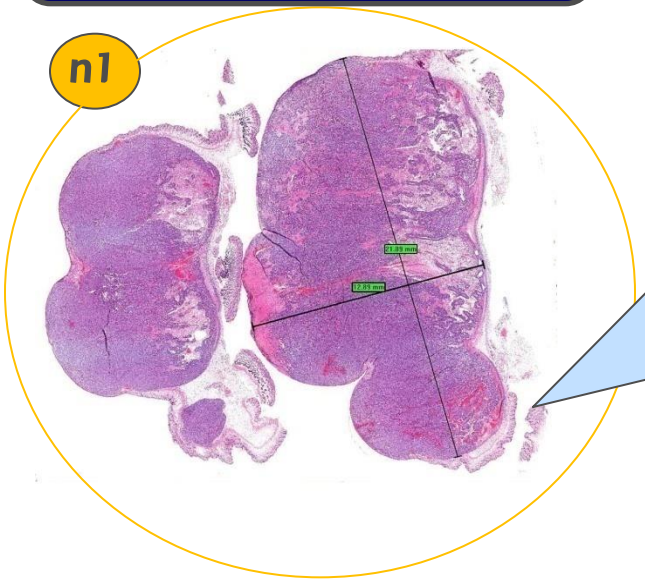
Drug tx group
MSB0052



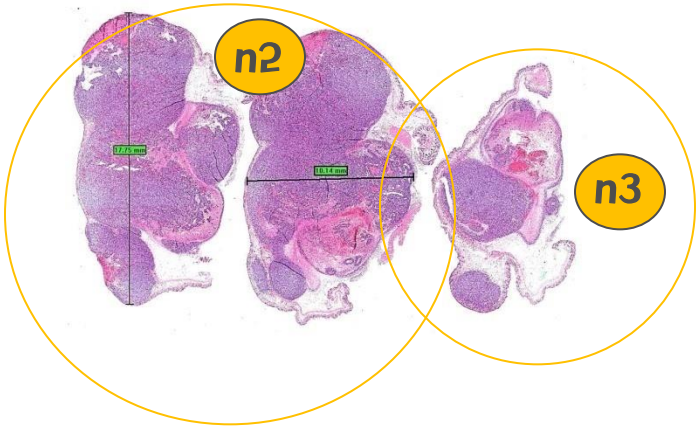
Macrophages

Pathological comparison after 15 days

Control group
With water n =1



15 days Tumor size larger , Penetrated the skin



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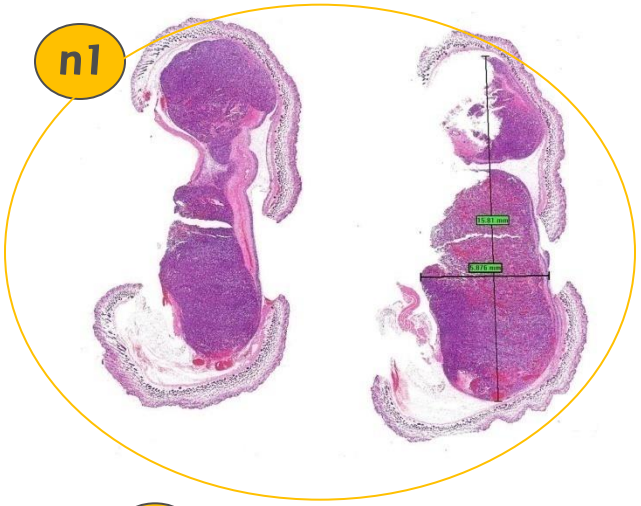
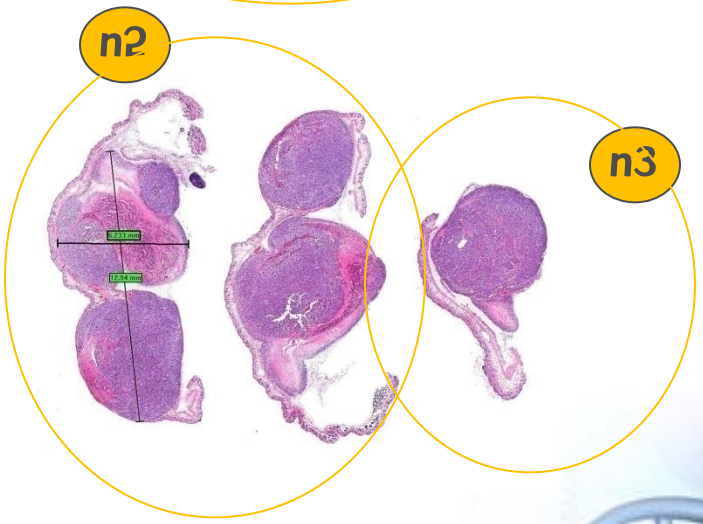
Drug tx group
With MSB0052 n = 1





Tumor volume growth. There was a difference in tumor volume between MSB0052 and control groups .

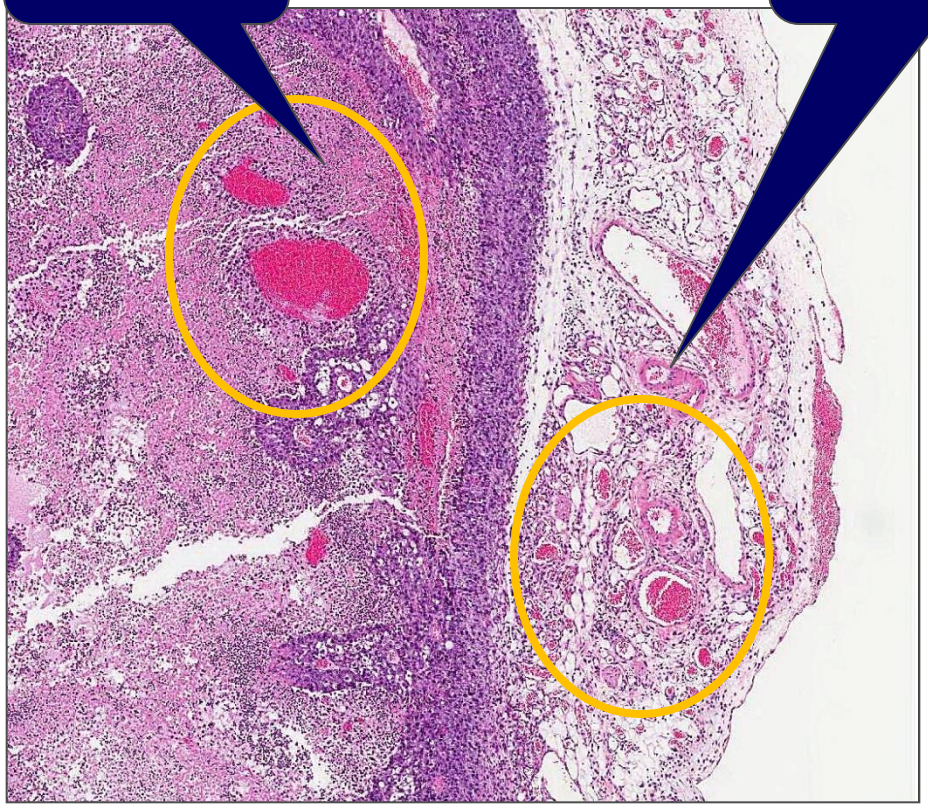
Micro in Control and MSB0052-15 D: Peritumoral reaction I

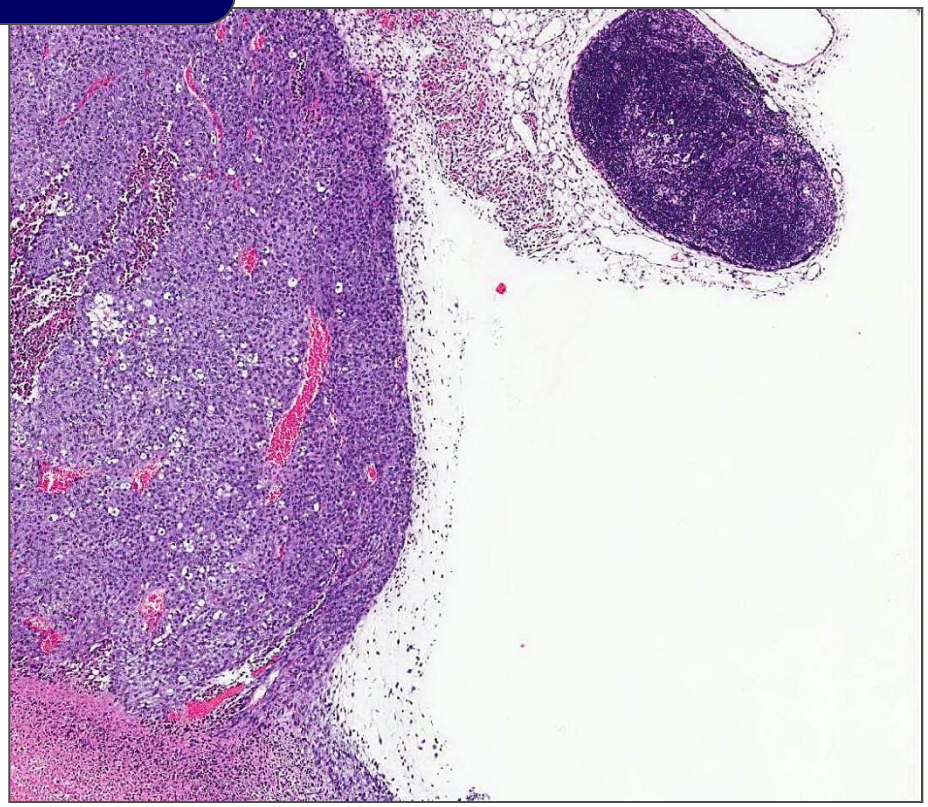
Necrotic changes

Control group

Angiogenesis

MSB0052 group



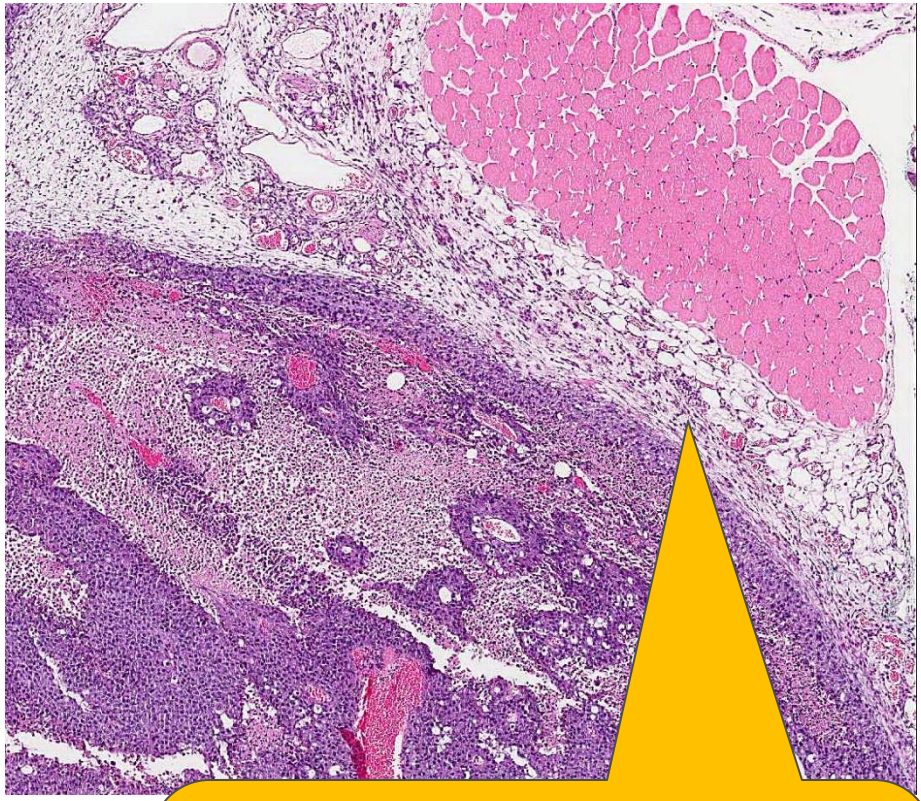


Contro group shows

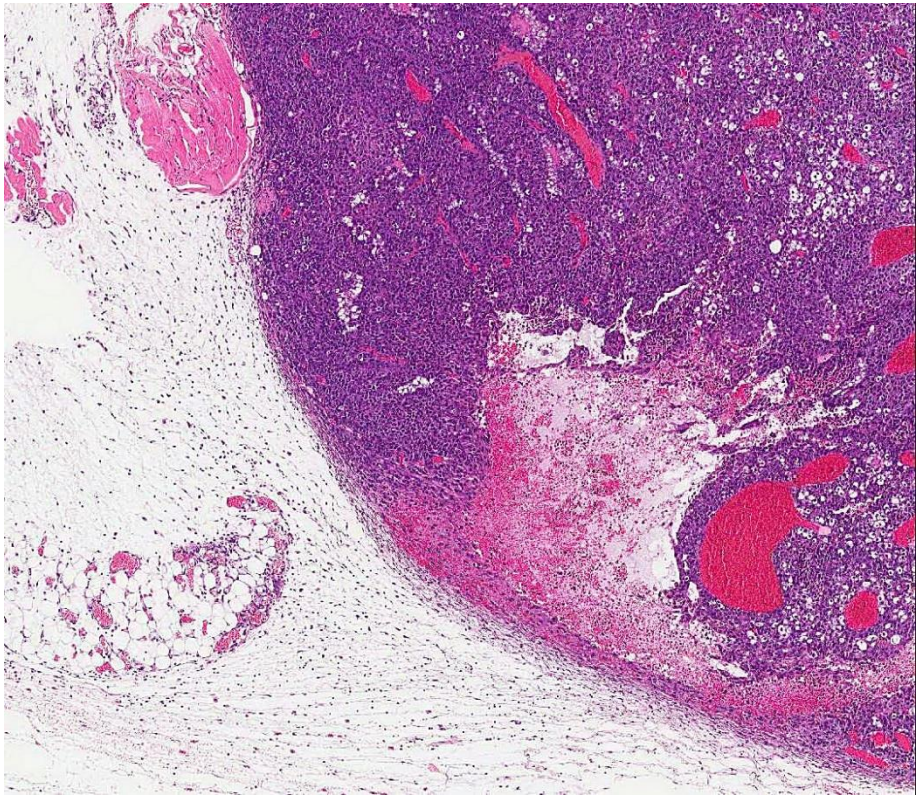
Increased fibroblastic proliferation **and** ***angiogenesis, necrotic change*** **in the tumor**

Micro in C-15 D: Peritumoral reaction II

Control group



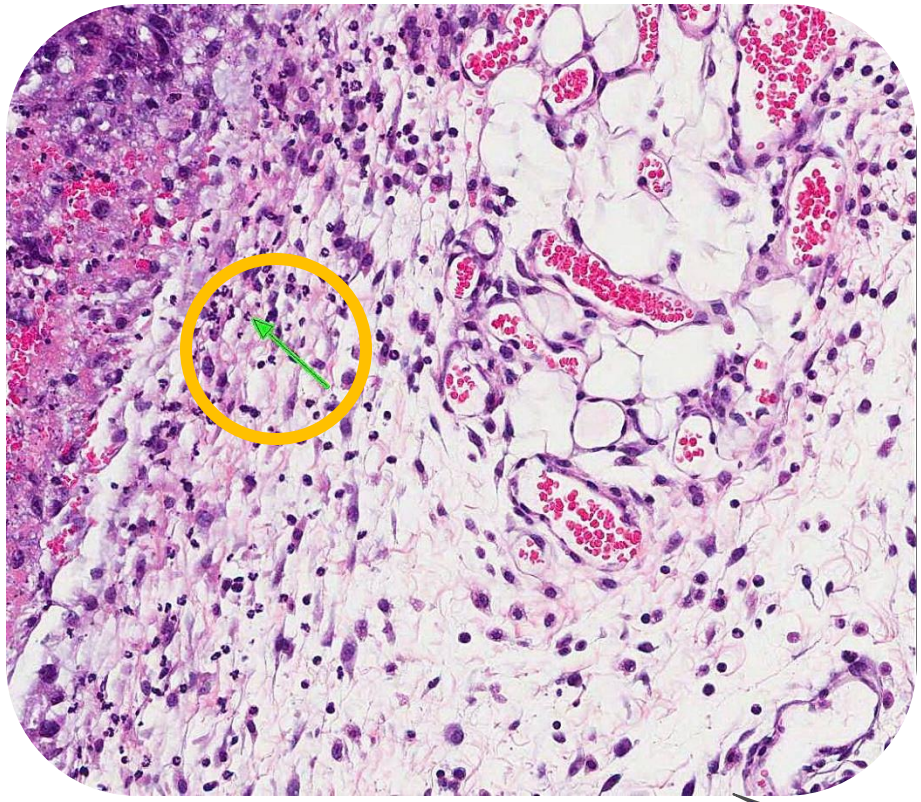
MSB0052 group



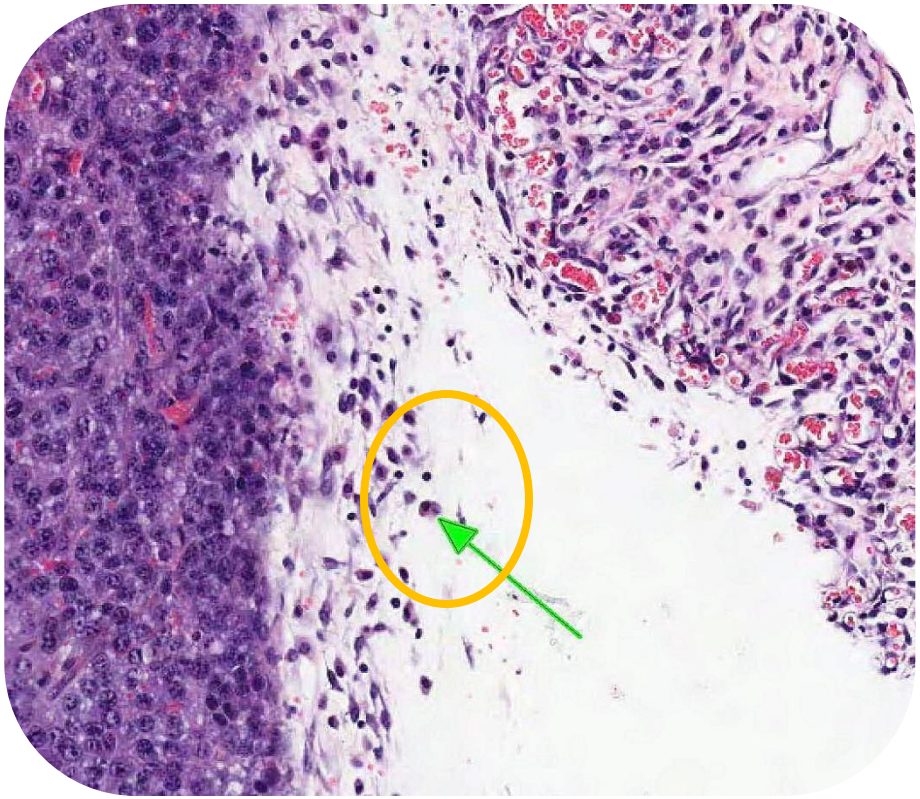
- ***Fibroblastic proliferation***
- ***angiogenesis***
- ***inflammatory reaction of neutrophil is prominent***

Micro in C-15 D: Peritumoral reaction II

Control group
With water



Drug tx group
With MSB00052



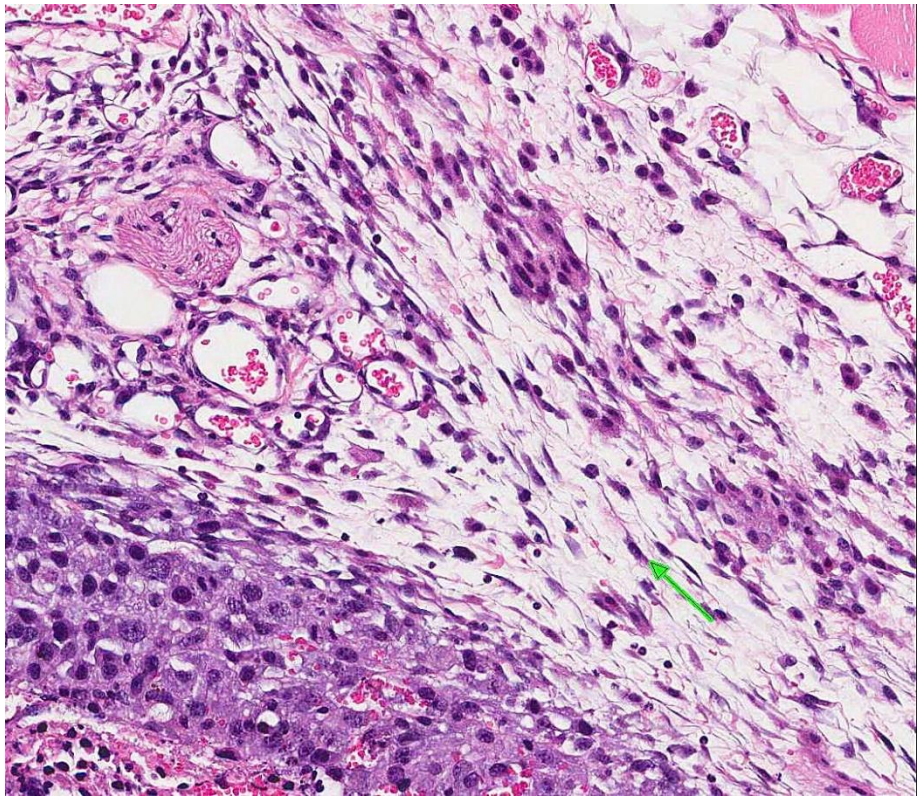
Increased fibroblastic proliferation and angiogenesis, neutrophil

neutrophil

macrophage

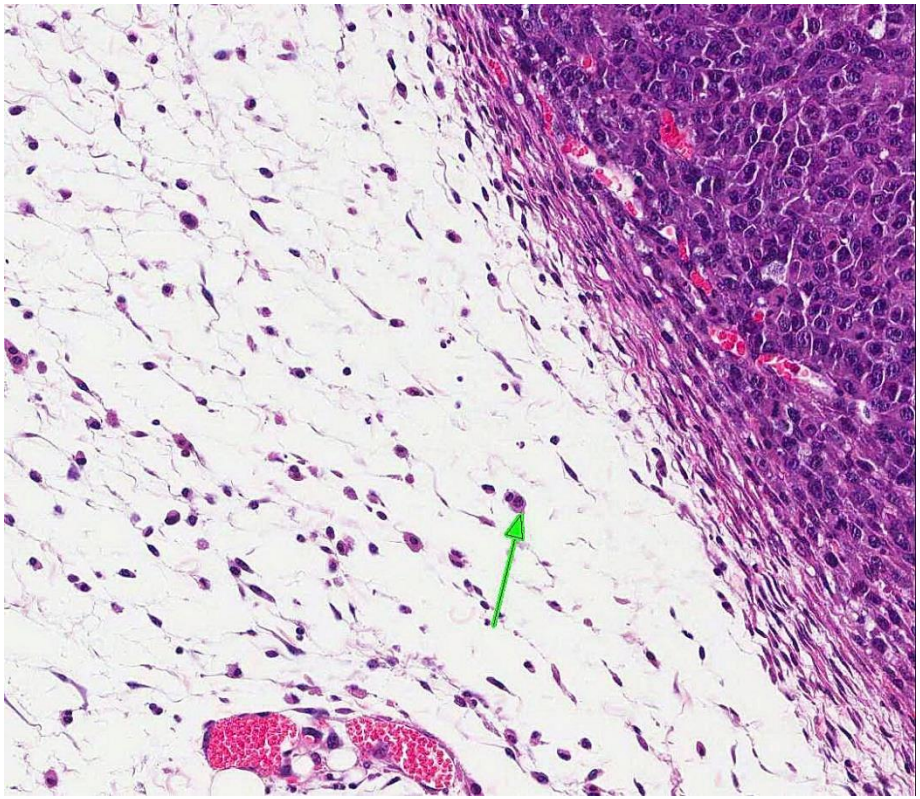
Micro in C-15 D: Peritumoral reaction II

Control group



fibroblastic proliferation and angiogenesis

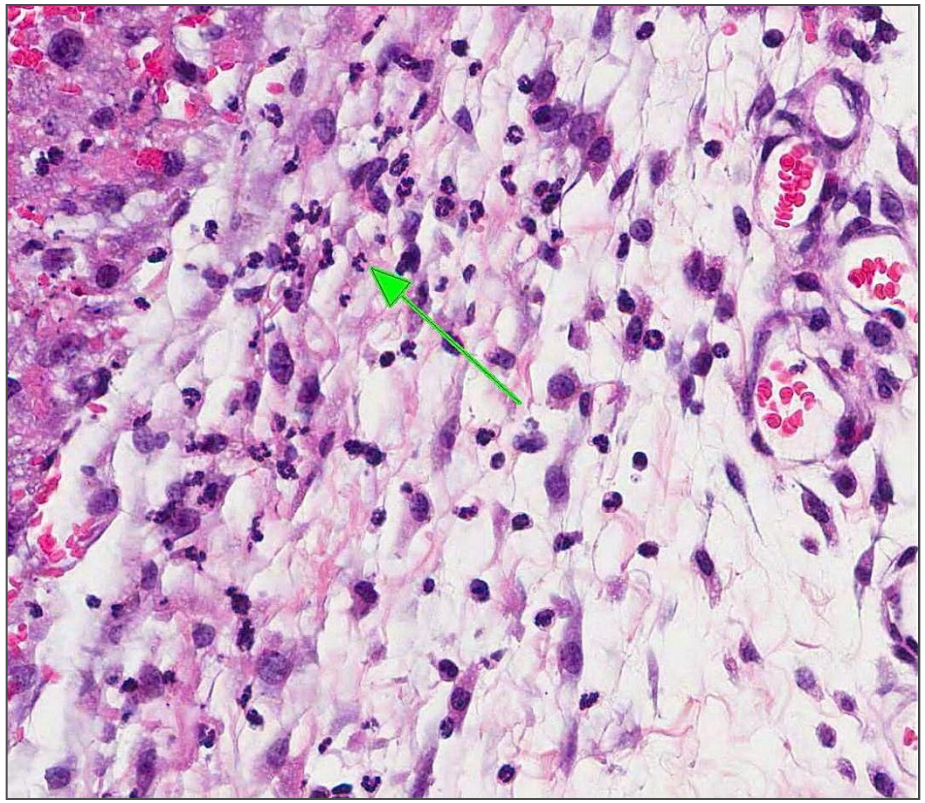
MSB0052 group



macrophage

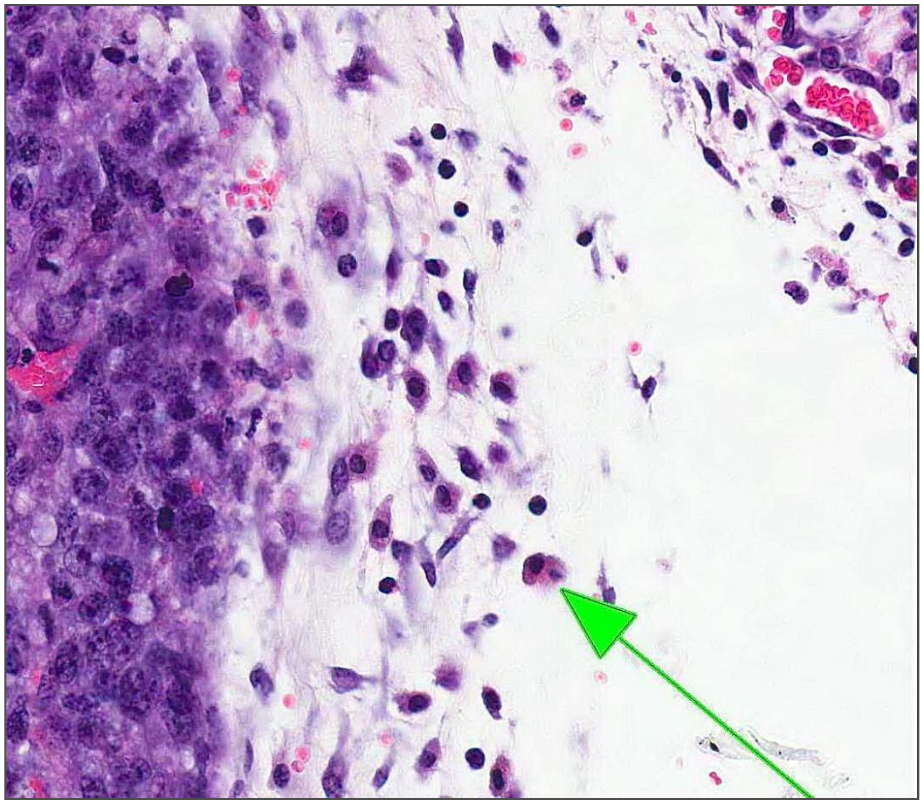
Micro in C-15 D: Peritumoral reaction III

Control group



Neutrophils

MSB0052 group



Macrophages

Comparison of composition- primo vessels

Experiment Design

Animal: Nude Mouse

↓

B16F10 cell 2.5×10^6/ml injection volume 1ml

↓

oral injection (every day)

↓

Start time 2006.28 →

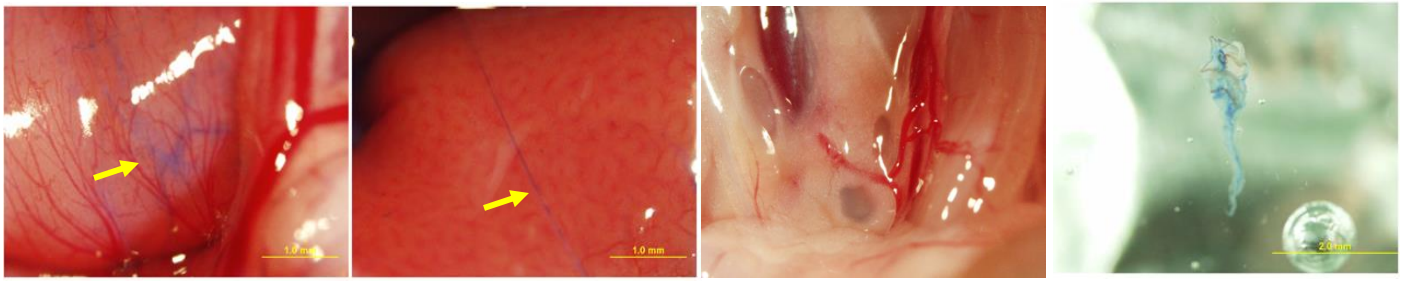
A-Group	B-Group	C-Group	D-Group
Native water o.inj	Control water o.inj	Test B0052 (50mg) o.inj	After tumor devolp B0052 (50mg) o.inj

↓

After sampling 17 days

Comparison of composition- primo vessels

A.



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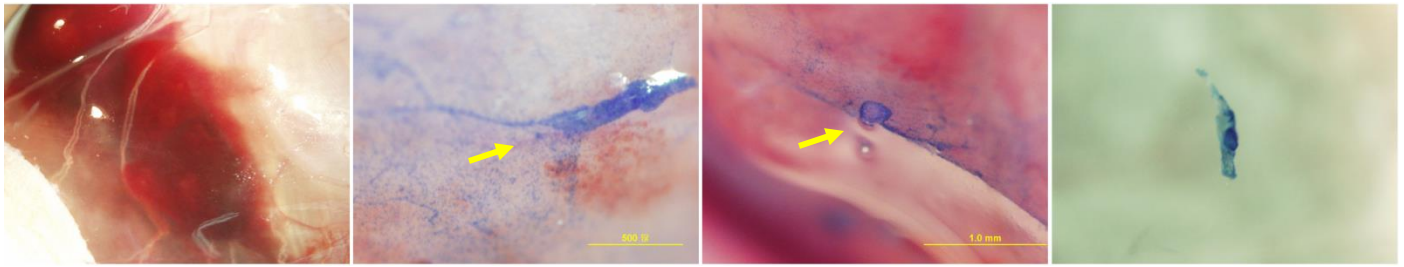
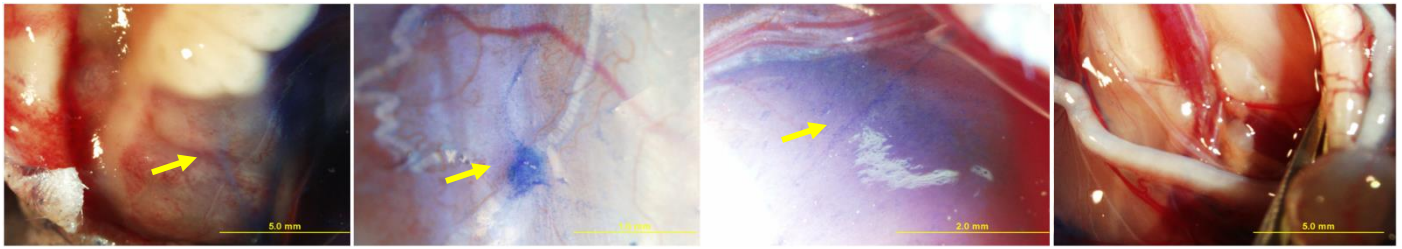
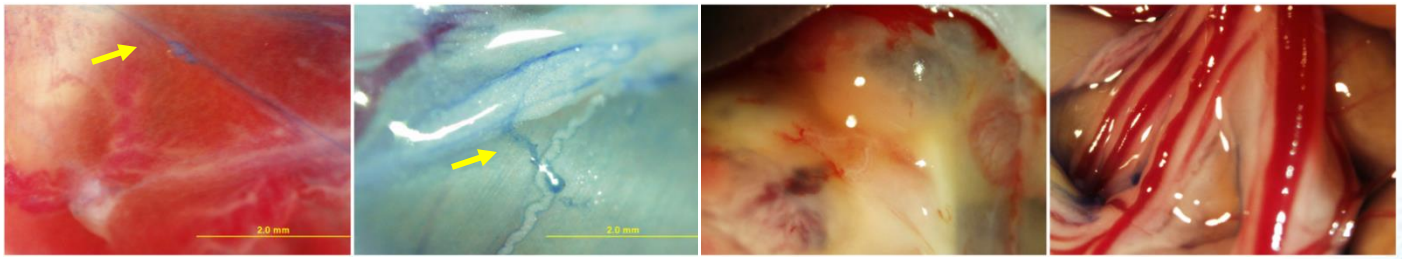


C.



D.



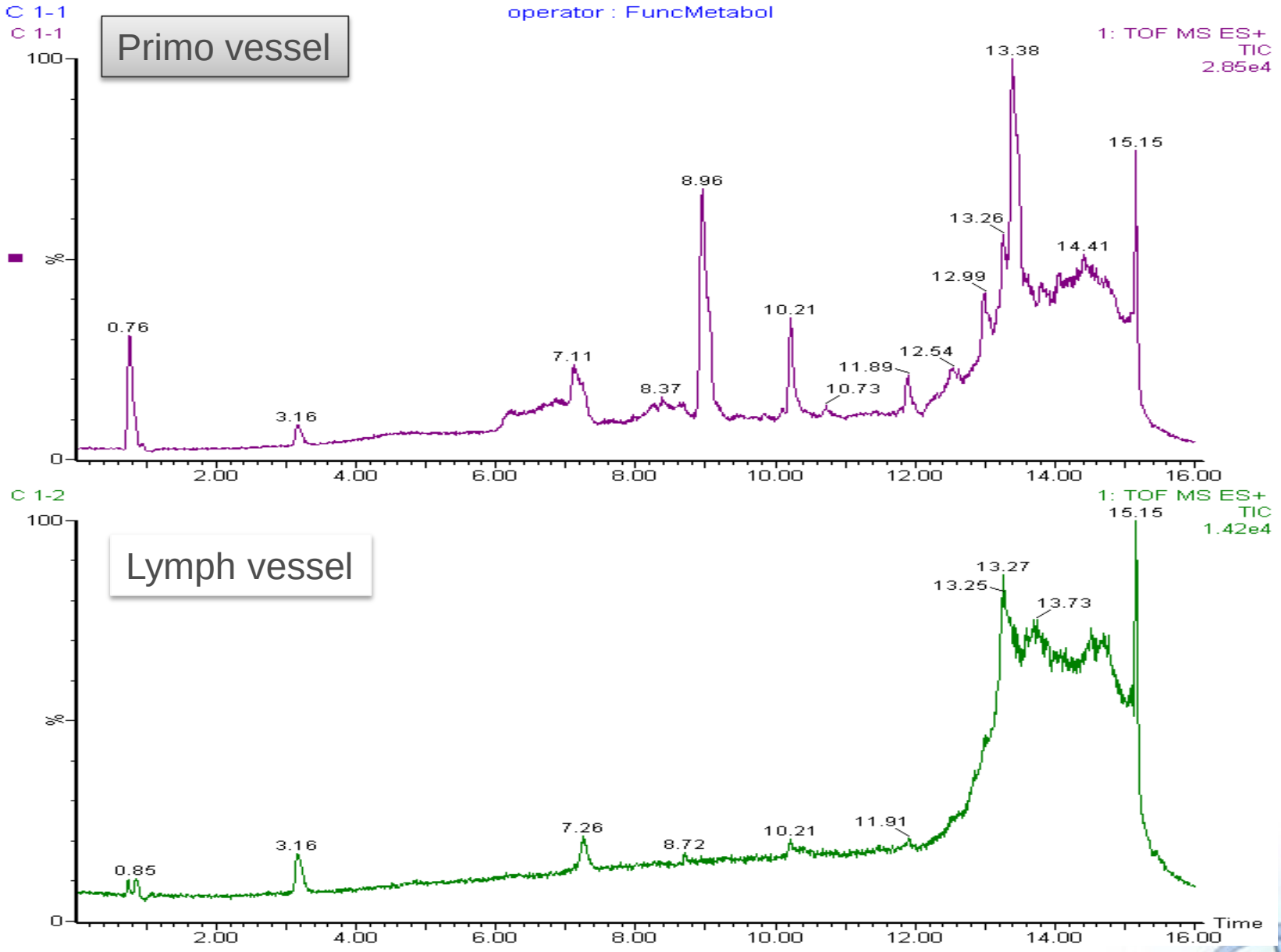
Condition of UPLC-Q-TOF-MS analysis

Waters ACQUITY UPLC ™ system (Waters, Milford, MA, USA)
Waters Q-TOF Premier (MicromassMSTechnologies, Manchester, UK)

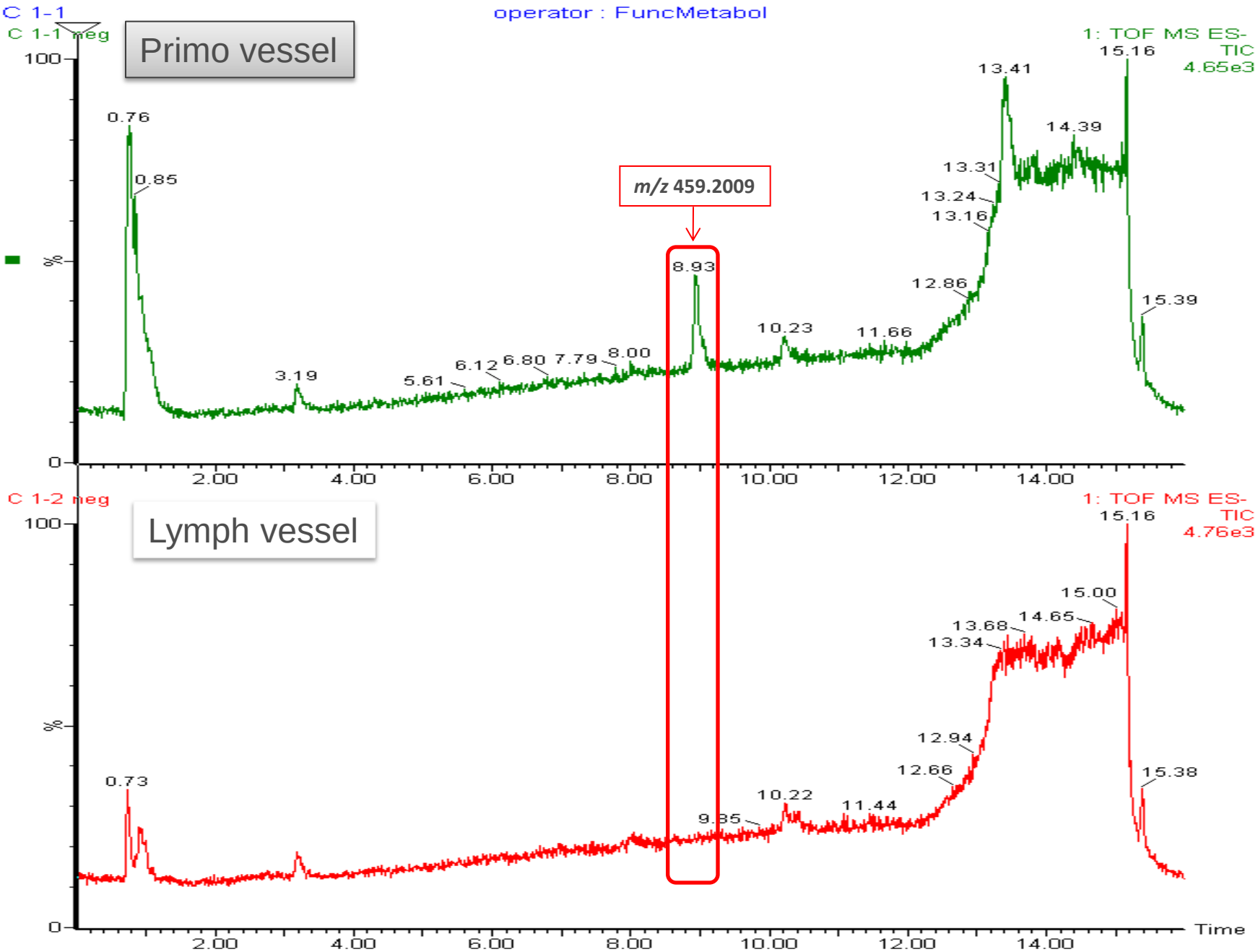
Run method parameters			
Run Time: 16.00 min			
Solvent Name A: 0.1%FA in Water Solvent Name B: 0.1% FA in ACN			
Time(min)	Flow Rate(mL)	%A	%B
1. initial	0.300	90	10
2. 1.00	0.300	90	10
3. 5.00	0.300	60	40
4. 11.00	0.300	30	70
5. 12.00	0.300	10	90
6. 14.00	0.300	10	90
7. 14.30	0.300	90	10
8. 16.00	0.300	90	10
Target Column Temperature: 35.0 C Column: Waters ACQUITY BEH C_{18} [100 x 2.1 mm i.d., 1.7 µm]			
Injection Volume (ul) - 5.00			

Instrument Configuration		
Mass range: 100 to 1500		
Polarity: Positive and Negative Capillary (kV): 2.5 (+), 3.0 (-)		
	(-)	(+)
Sample Cone (V)	30.0	25.0
Extraction Cone (V)	2.5	2.0
Desolvation Temp (℃)	200.0	200.0
Source Temp (℃)	120.0	100.0
Ion Energy (V)	1.0	1.0
Collision Energy	5.0	5.0

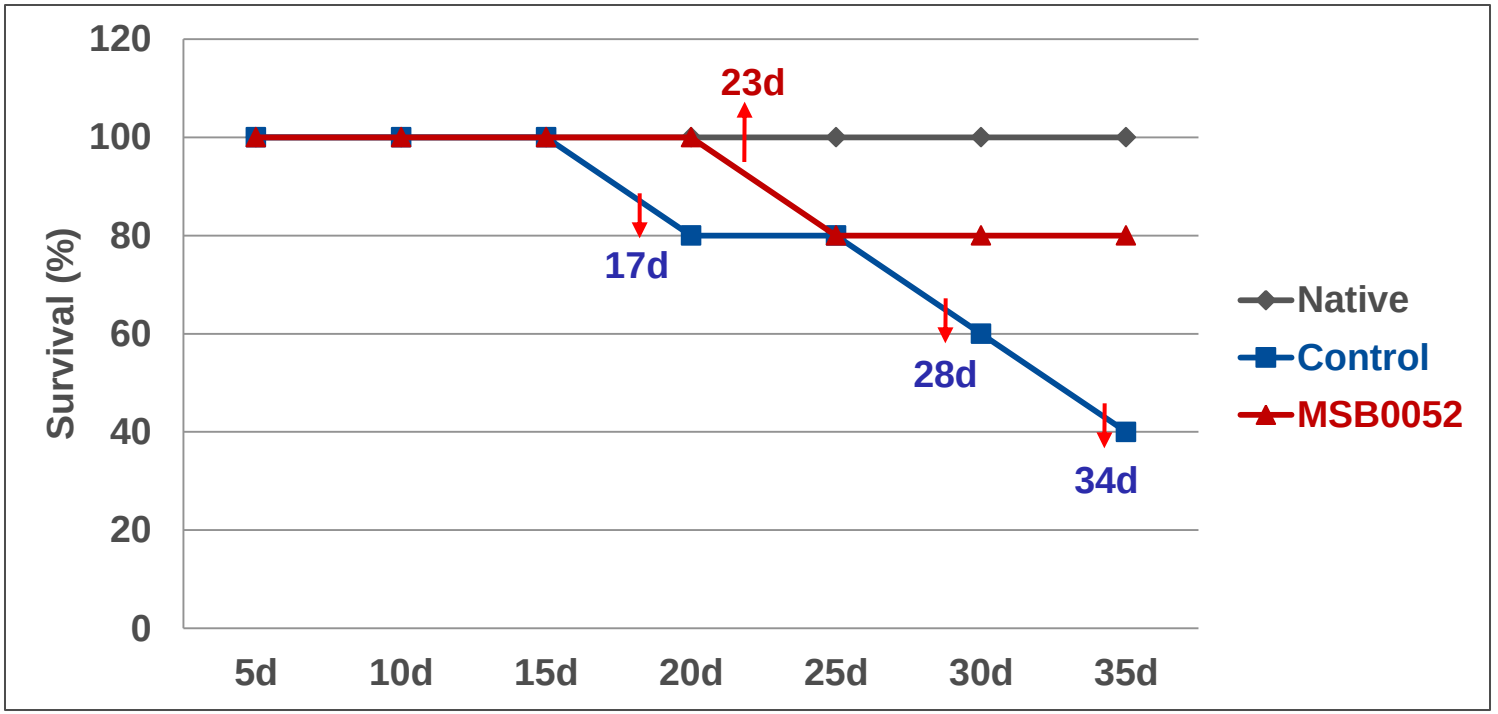
UPLC-Q-TOF mass analysis_ Total chromatogram at positive mode



UPLC-Q-TOF mass analysis_ Total chromatogram at negative mode



Survival curve of tumor-bearing mice



Survival curve for tumor-bearing mice. MSB0052-treated mice was also found when compared with the control groups.

Activity between two groups

Conclusion

MSB0052 has Anti-tumoral effect on murine melanoma

Strenghten the immune reaction like macrophage

inhibitory effect on angiogenesis

inhibitory effect on inflammatory reaction like neutrophil

Acknowledgment:

- Baatartsogt .O, (Hankyong National University).
- Jung Sun Yoo*, (Seoul National University).
- Hee Jae Ju. (Ajou University).
- Kwang Sup Soh*, (Seoul National University).
- Mi Son Chun, (Ajou University).
- Kang Duk Choi, (Hankyong National University).
- Choong Hwan Lee, (Konkuk University).
- Ji Young Kim, (konkuk university).
- Namhyun Jung (Korea university)
- Il Young Han, (sonyun biophics).


Thank you for your attention!