Faraday PHARMACEUTICALS

Iodide reduces cachexia in a BALB/c CT26 mouse tumor model

Michael A. Insko

December 7th, 2019

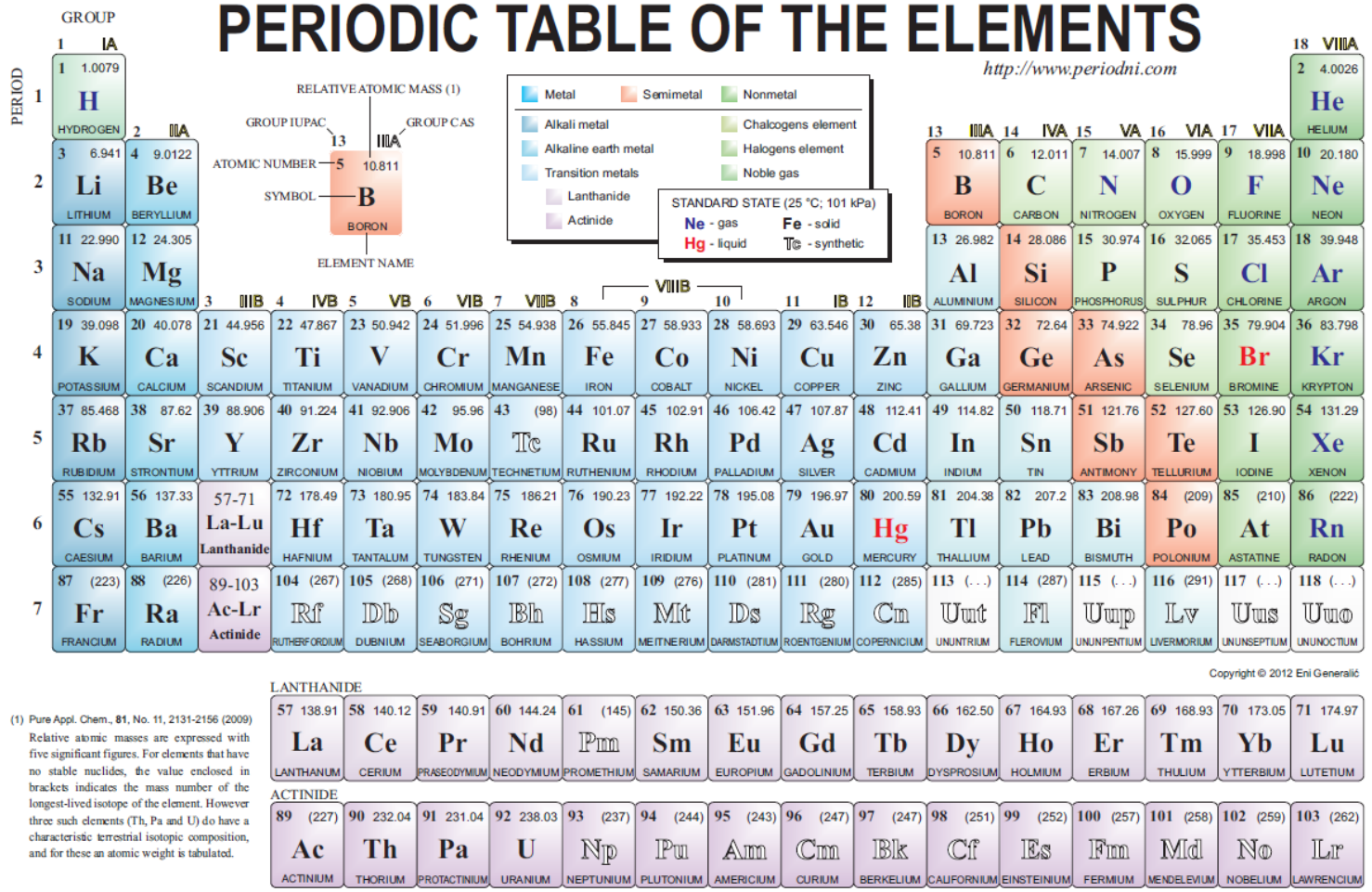
12th International Conference on Cachexia, Sarcopenia & Muscle Wasting

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Introduction to ERA's and the MOA of Iodide

- Elemental Reducing Agents (ERA's)
- Mechanism of Action (MOA) of iodide

Elemental Reducing Agents



Sulfide

$Na_2S \rightarrow Na^+ + S^-$

Selenide

$Na_2Se \rightarrow Na^+ + Se^-$

Iodide

$NaI \rightarrow Na^+ + I^-$

Faraday PHARMACEUTICALS

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Elemental Reducing Agents

- Sulfide (S^-) has been shown to prevent death in models of lethal hypoxia (1), increase survival during hemorrhagic shock (2) and also prevent cardiac and skeletal muscle damage in models of ischemia/reperfusion (3, 4).
  1. Blackstone, E. and M.B. Roth, Suspended animation-like state protects mice from lethal hypoxia. Shock, 2007. 27(4): p. 370-2.
  2. Morrison, M.L., et al., Surviving blood loss using hydrogen sulfide. J Trauma, 2008. 65(1): p. 183-8.
  3. Donnarumma, E., R.K. Trivedi, and D.J. Lefer, Protective Actions of H2S in Acute Myocardial Infarction and Heart Failure. Compr Physiol, 2017. 7(2): p. 583-602.
  4. Langston, J.W. and C.F. Toombs, Defining the minimally effective dose and schedule for parenteral hydrogen sulfide: long-term benefits in a rat model of hindlimb ischemia. Med Gas Res, 2015. 5: p. 5.

- Selenide (Se^-) has been shown to prevent cardiac damage in I/R models
  5. Iwata, A., et al., Selenide Targets to Reperfusing Tissue and Protects It From Injury. Crit Care Med, 2015. 43(7): p. 1361-7.

- Sulfide and selenide degrade rapidly in the presence of oxygen and are difficult to manufacture.
- Sulfide gives off its characteristic “rotten egg” smell when administered intravenously (6, 7)
  6. Insko, M.A., et al., Detection of exhaled hydrogen sulphide gas in rats exposed to intravenous sodium sulphide. Br J Pharmacol, 2009. 157(6): p. 944-51.
  7. Toombs, C.F., et al., Detection of exhaled hydrogen sulphide gas in healthy human volunteers during intravenous administration of sodium sulphide. Br J Clin Pharmacol, 2010. 69(6): p. 626-36.

- Iodide (I^-) can reduce infarct size in mouse (8), rat & pig models (9) of I/R.
  8. Iwata, A., M.L. Morrison, and M.B. Roth, Iodide protects heart tissue from reperfusion injury. PLoS One, 2014. 9(11): p. e112458.
  9. Morrison, M.L., et al., Iodide Improves Outcome After Acute Myocardial Infarction in Rats and Pigs. Crit Care Med, 2018. 46(11): p. e1063-e1069.

- In results recently presented at the 2019 American Heart Association meeting, we showed that administration of FDY-5301 (**sodium iodide**) decreased median infarct size from 14.9% (placebo) to 8.5% (2 mg/kg FDY-5301) (not statistically significant).

Iodide modulates the immune response

- Iodide can inhibit neutrophil chemotaxis (10) and is an effective therapeutic for the treatment of: erythema nodosum & nodular vasculitis (11), as well as acute febrile neutrophilic dermatosis (Sweet's Syndrome) (12).

10. Honma, K., et al., Potassium iodide inhibits neutrophil chemotaxis. Acta Derm Venereol, 1990. 70(3): p. 247-9.
11. Horio, T., et al., Treatment of acute febrile neutrophilic dermatosis (Sweet's Syndrome) with potassium iodide. Dermatologica, 1980. 160(5): p. 341-7.
12. Cohen, P.R., Sweet's syndrome--a comprehensive review of an acute febrile neutrophilic dermatosis. Orphanet J Rare Dis, 2007. 2: p. 34.

- Iodide markedly reduces gene expression levels of inflammatory cytokines in a mouse model of SDS induced inflammation (13)

13. Hayashi, S., Y. Hamasaki, and A. Hatamochi, The anti-inflammatory effects of potassium iodide in SDS-induced inflammatory murine skin. Journal of Dermatological Science, 2017. 86(2): p. e16-e17.

- Iodide reduces inflammation in rat model of carageenan induced inflammation (14)

14. Shenoy, R. and P. Patil, Effects of sodium iodide on inflammation and its interaction with aspirin and mefenamic acid in albino rats. Vol. 3. 2007.

- Iodide has recently been shown to reduce markers of intramuscular inflammation (mouse I/R) and prevent muscle cachexia (15)

15. Insko, M.A., Iodide reduces intramuscular inflammation following hind limb ischemia in mice, in 12th international SCWD conference on cachexia, sarcopenia and muscle wasting. 2019: Berlin, Germany.

Iodide catalytically disproportionates H_2O_2

- The catalytic disproportionation of H_2O_2 by iodide was formally characterized and empirically validated by Abel (16) in 1928 and subsequently verified by Liebhafsky in 1932 (17).

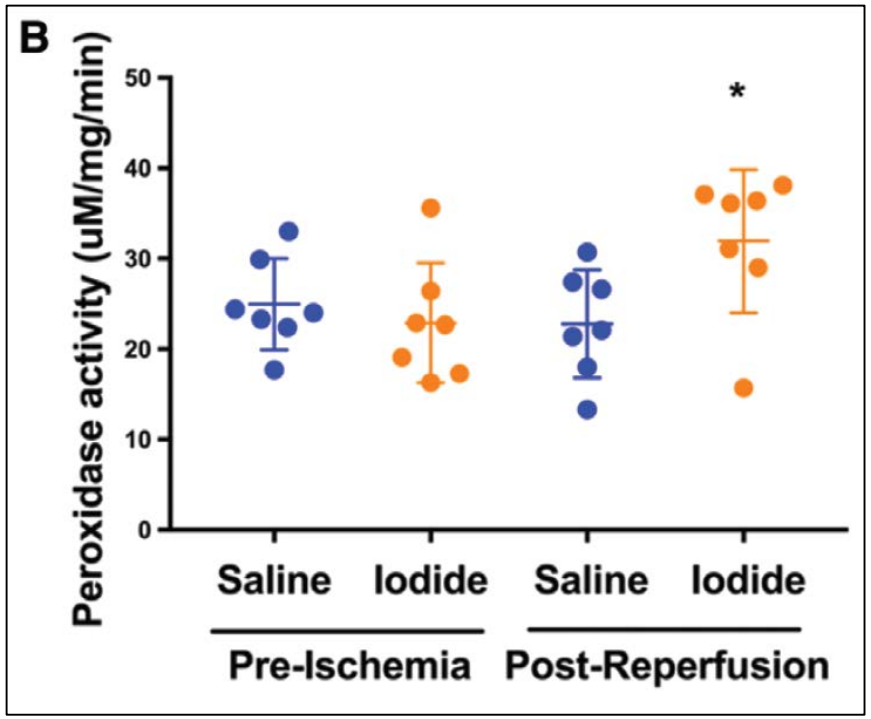
16. Abel, E., Über das Reaktionenspiel zwischen Wasserstoffsuperoxyd, Jod und Jodion.". Zeitschrift für physikalische Chemie, 1928. 136: p. 161-182.
17. Liebhafsky, H.A., THE CATALYTIC DECOMPOSITION OF HYDROGEN PEROXIDE BY THE IODINE-IODIDE COUPLE AT 25°. Journal of the American Chemical Society, 1932. 54(5): p. 1792-1806.

$$I^- + H_2O_2 \rightarrow IO^- + H_2O$$

$$IO^- + H_2O_2 \rightarrow I^- + H_2O + O_2$$

Net reaction:

$$\mathbf{2\ H_2O_2 \rightarrow 2\ H_2O + O_2}$$



- In a pig model of cardiac I/R we demonstrated that a 1 mg/kg i.v. bolus of sodium iodide (given 5 min prior to reperfusion) significantly increases peroxidase activity (9)
- We have evidence of similar peroxidase activity in human plasma spiked with sodium iodide to mimic the C_{max} following a 0.5, 1, or 2 mg/kg i.v. bolus (unpublished)

Faraday PHARMACEUTICALS

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Iodide vs 'sacrificial' antioxidants

Iodide	Vitamin C	Tocopherols
Catalytically disproportionates H_2O_2, therefore reducing subsequent emergence of other ROS such as hydroxyl (·OH) and superoxide (O_2^-) free radicals (16, 17)	Vitamin C and E (and analogues) are converted from anti-oxidant to pro-oxidant over time (19, 20)	
Endogenous thyroid hormones are de-iodinated in response to injury releasing free iodide therefore increasing availability (18)	endogenous levels are depleted early during critical illness (21)	Vitamin E concentrations in critically ill patients are significantly reduced (24)
Half life following i.v. administration is approximately 8 hours (healthy volunteers) to ~20 hours (AMI patients) (unpublished)	Half life following i.v. administration ~ 30 minutes (22) to 1.87 hr (23)	Vitamin E delta-tocotrienol (VEDT) Half life of 1.7 – 5.9 hr following p.o. administration (25)

18. Chatzitomaris, A., et al., Thyroid Allostasis-Adaptive Responses of Thyrotropic Feedback Control to Conditions of Strain, Stress, and Developmental Programming. Front Endocrinol (Lausanne), 2017. 8: p. 163.
19. Chakraborthy, A., et al., Antioxidant and pro-oxidant activity of Vitamin C in oral environment. Indian J Dent Res, 2014. 25(4): p. 499-504.
20. Tafazoli, S., J.S. Wright, and P.J. O'Brien, Prooxidant and antioxidant activity of vitamin E analogues and troglitazone. Chem Res Toxicol, 2005. 18(10): p. 1567-74.
21. Goode, H.F., et al., Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. Crit Care Med, 1995. 23(4): p. 646-51.
22. Hickey, S., Ascorbate: The Science of Vitamin C. 2004: Lulu Press. 264.
23. Nielsen, T.K., et al., Elimination of ascorbic acid after high-dose infusion in prostate cancer patients: a pharmacokinetic evaluation. Basic Clin Pharmacol Toxicol, 2015. 116(4): p. 343-8.
24. Bertrand, Y., et al., Differences in tocopherol-lipid ratios in ARDS and non-ARDS patients. Intensive Care Med, 1989. 15(2): p. 87-93.
25. Mahipal, A., et al., Pharmacokinetics and safety of vitamin E delta-tocotrienol after single and multiple doses in healthy subjects with measurement of vitamin E metabolites. Cancer Chemother Pharmacol, 2016. 78(1): p. 157-65.

BALB/c CT26 mouse tumor model

- Implications of reactive oxygen species (ROS) in cachexia
- BALB/c tumor model design and results

Role of oxidative stress in cachexia

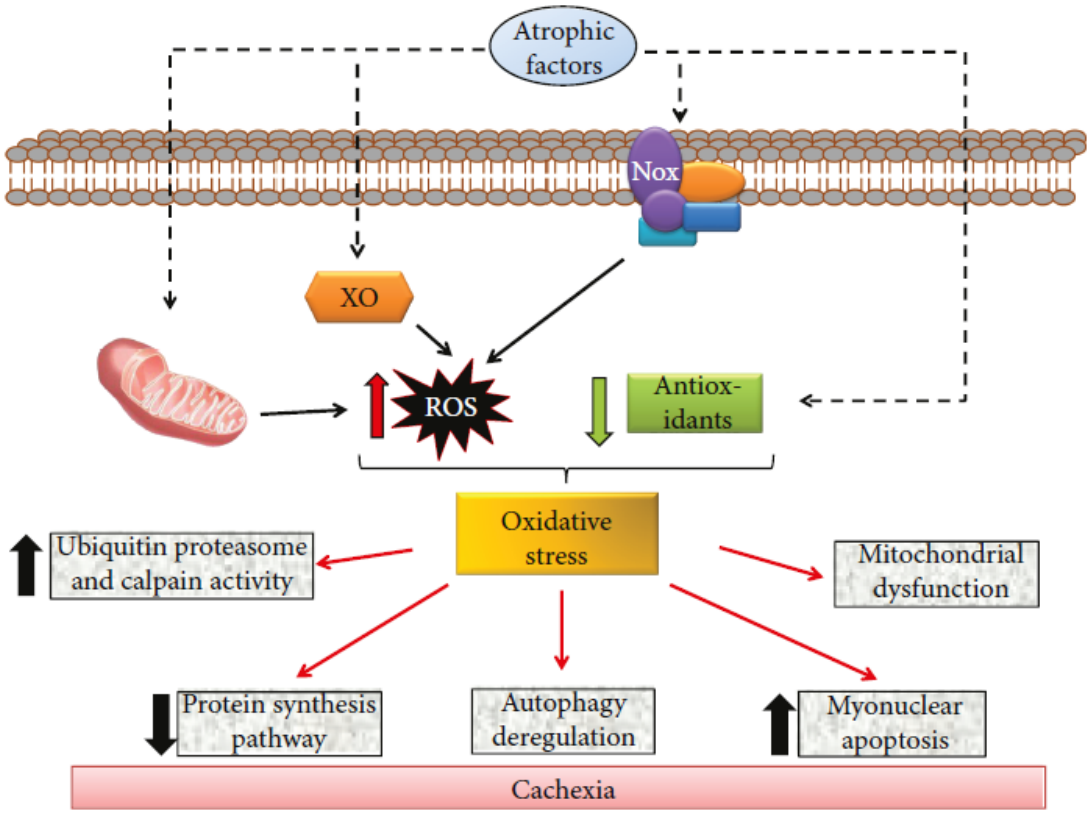


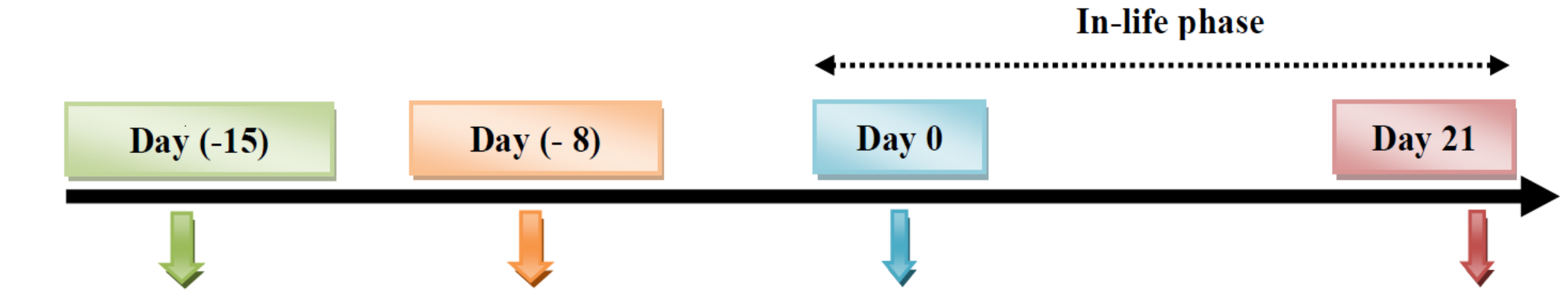
FIGURE 2: Molecular mechanisms involved in cachexia are modulated by oxidative stress. Atrophic factors can generate oxidative stress in skeletal muscle by the activation of different sources of reactive oxygen species, such as the mitochondria, xanthine oxidase (XO), and NADPH oxidase complex with Nox subunit, in addition to the decrease in antioxidant species. Oxidative stress is able to produce mitochondrial dysfunction, increase ubiquitin proteasome system activity, increase myonuclear apoptosis, decrease the protein synthesis pathway, and deregulate autophagy, all of which are involved in cachexia-skeletal muscle atrophy.

Abrigo, J., et al., Role of Oxidative Stress as Key Regulator of Muscle Wasting during Cachexia. Oxid Med Cell Longev, 2018. 2018: p. 2063179.

Faraday PHARMACEUTICALS

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Model Design



Tumor volume (mm^3): 0, 3, 6, 9, 12, 15, 18 & 21.

End Points:

Body weight (% body weight loss),
Daily food intake
Clinical observations
Blood sampling – Biochemical & cytokine analysis
Organ & Muscle weight
Histology of muscle

Treatment Groups

Tumor type	Cell line	Tumor volume (Study initiation)	No. of Animals	Treatment	Dose	Route	Schedule
Murine colorectal carcinoma	T26	When TV reaches 100mm^3	10	Normal	-	-	-
			10+3	Vehicle Control 0.5% CMC	-	p.o	QD x 3 weeks
			10+3	FDY-5301	2mg/kg	i.v	QD x 3 weeks
			10	Bucindolol	2mg/kg	p.o	QD x 3 weeks
			10+3	FDY-5301	40µg/day slow release via Alzet osmotic pump (flow rate-0.11µL/h)	s.c	20 days

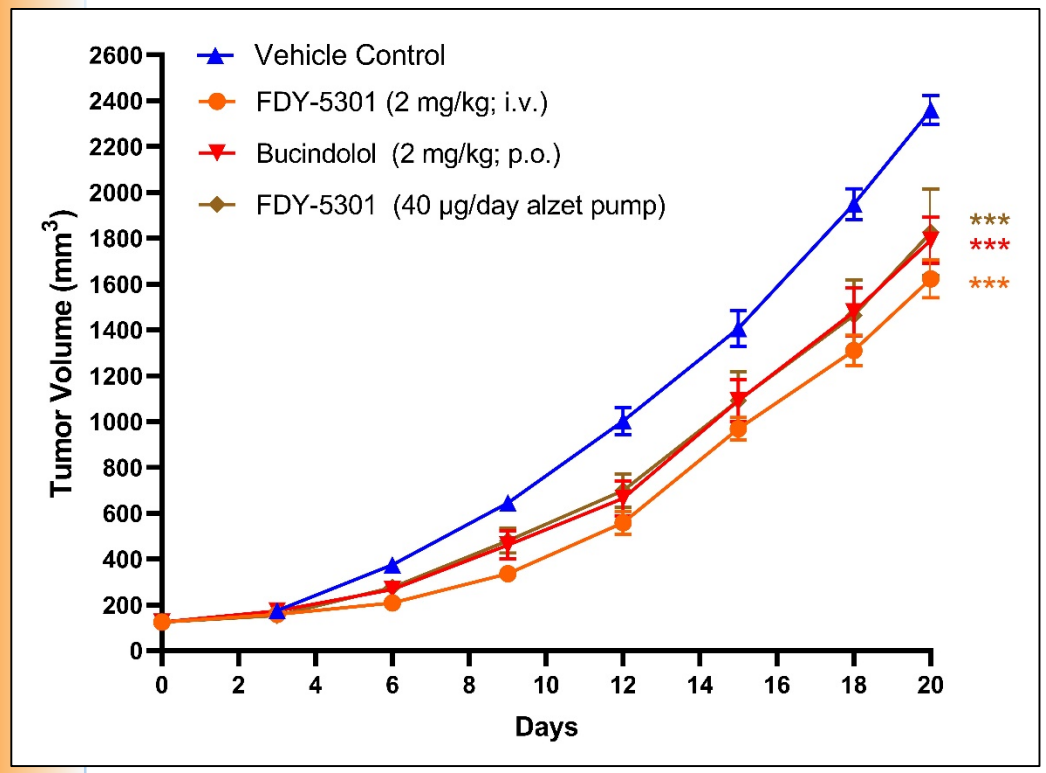
- We chose a beta blocker as a positive control as they have shown promise in preventing cachexia related to cancer (27) and heart failure (28).

27. Stewart Coats, A.J., et al., Espindolol for the treatment and prevention of cachexia in patients with stage III/IV non-small cell lung cancer or colorectal cancer: a randomized, double-blind, placebo-controlled, international multicentre phase II study (the ACT-ONE trial). J Cachexia Sarcopenia Muscle, 2016. 7(3): p. 355-65.
28. Clark, A.L., et al., Effect of beta-adrenergic blockade with carvedilol on cachexia in severe chronic heart failure: results from the COPERNICUS trial. J Cachexia Sarcopenia Muscle, 2017. 8(4): p. 549-556.

Note: **On day 14, 1 h post dosing, blood sampling will be carried out - 3 animals from group 2, 3 & 5.*

Mean Tumor Volume (mm^3) & Tumor Growth Kinetics

- FDY-5301 & Bucindolol inhibit tumor growth by 23 - 31%

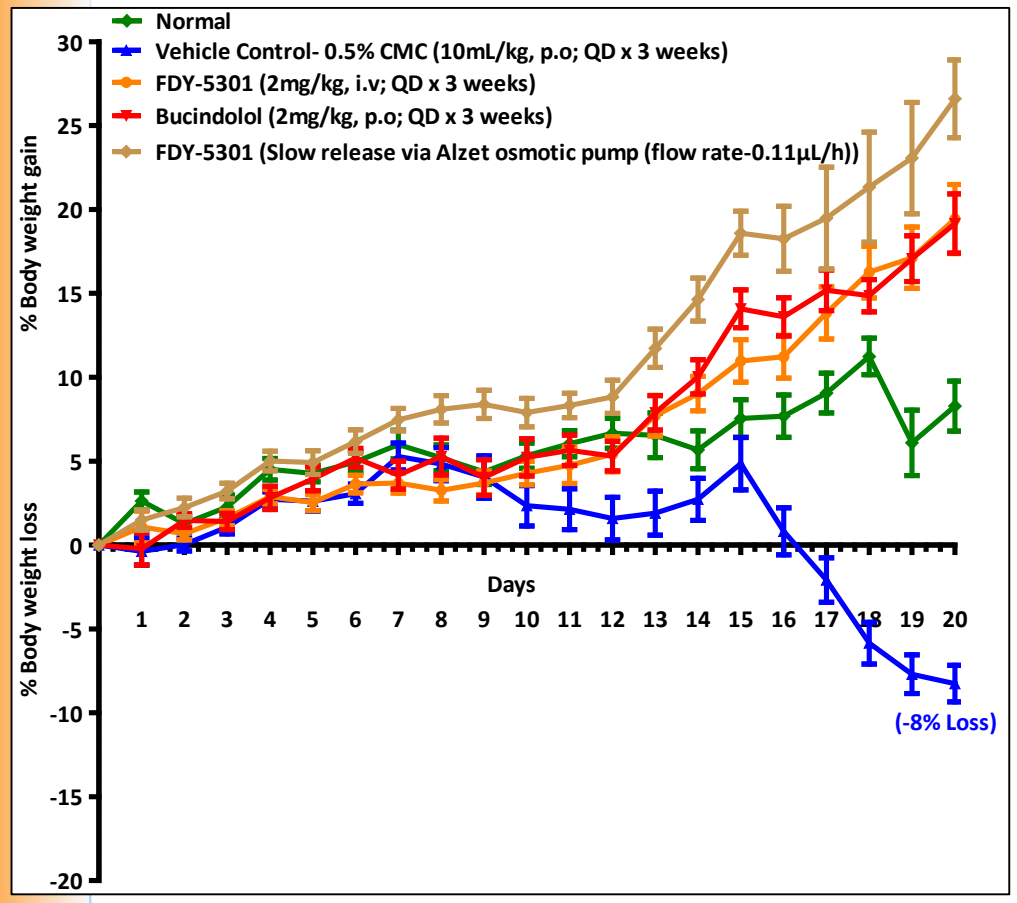


Group	**Treatment** & Dose	Tumor volume (mm^3) day 20 Mean ± SEM	% Tumor growth inhibition on day 20
Group -1	Normal	-	-
*Group -2	Vehicle control 0.5 % CMC (10mL/kg, p.o; QD x 3 weeks)	2361 ± 64	-
*Group -3	FDY-5301 (2mg/kg, i.v; QD x 3 weeks)	1623 ± 83	31***
Group -4	Bucindolol (2mg/kg, p.o; QD x 3 weeks)	1791 ± 100	24***
*Group -5	FDY-5301 (Slow release via Alzet osmotic pump flow rate-0.11µL/h)	1825 ± 189	23***

Values are expressed as Mean ± SEM of 10-13 animals in each group
Statistical analysis carried out by Two-way ANOVA followed by Bonferroni post tests using Graph Pad Prism (Version.5)
*** p<0.001 when respective test groups (FDY-5301 & Bucindolol) were compared with vehicle control group.

% Mean Body Weight Change

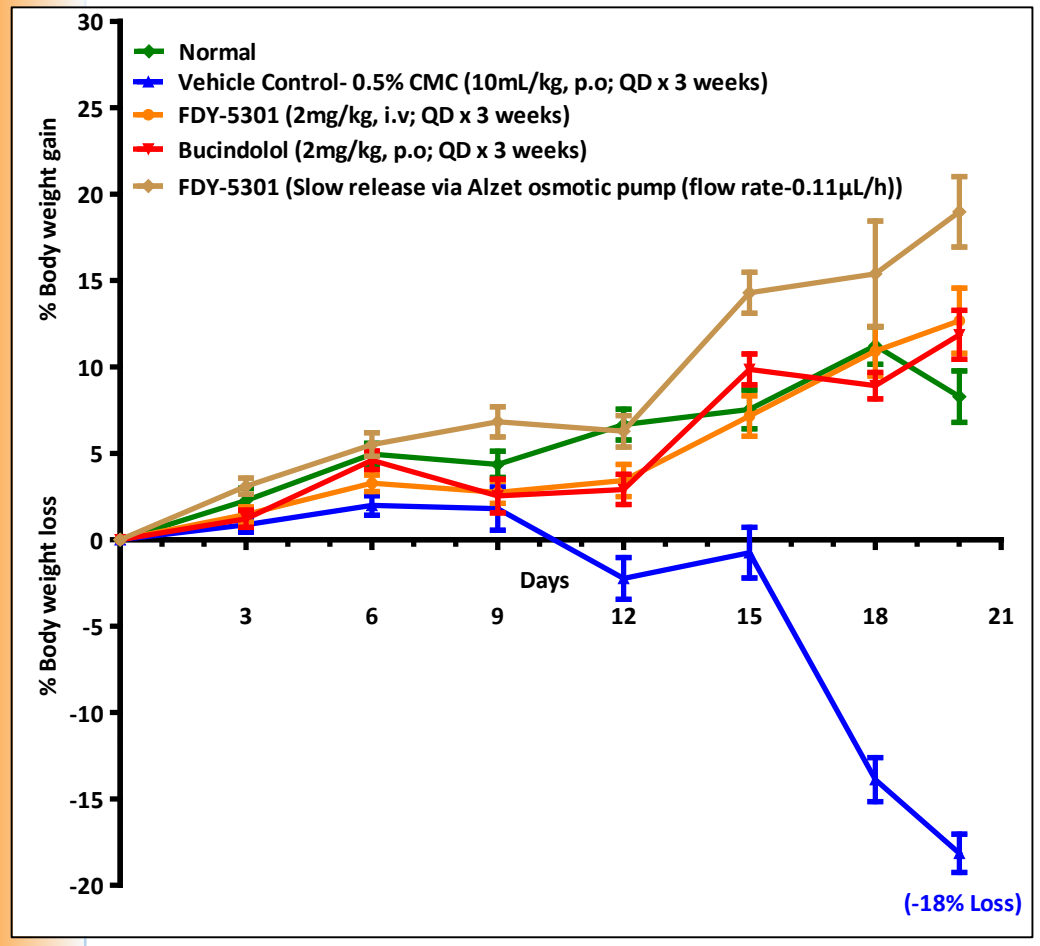
- FDY-5301 & Bucindolol help maintain body weight



Group	% Mean body weight change (Day20)
Group -1 – Normal	8% gain
Group -2 – Vehicle control- 0.5 % CMC (10mL/kg, p.o; QD x 3 weeks)	-8% loss
Group -3 - FDY-5301 (2mg/kg, i.v; QD x 3 weeks)	19% gain
Group -4 - Bucindolol (2mg/kg, p.o; QD x 3 weeks)	19% gain
Group -5 - FDY-5301 (Slow release via Alzet osmotic pump flow rate-0.11µL/h)	27% gain

Values are expressed as Mean ± SEM of 10-13 animals in each group.
Based on cage side observations, there were no visible signs of abnormal behavior or clinical symptoms in any of the treated groups

Percentage change in tumor free body weight of Balb/c mice treated with FDY-5301 & Bucindolol in animals bearing subcutaneous CT26 syngeneic tumor



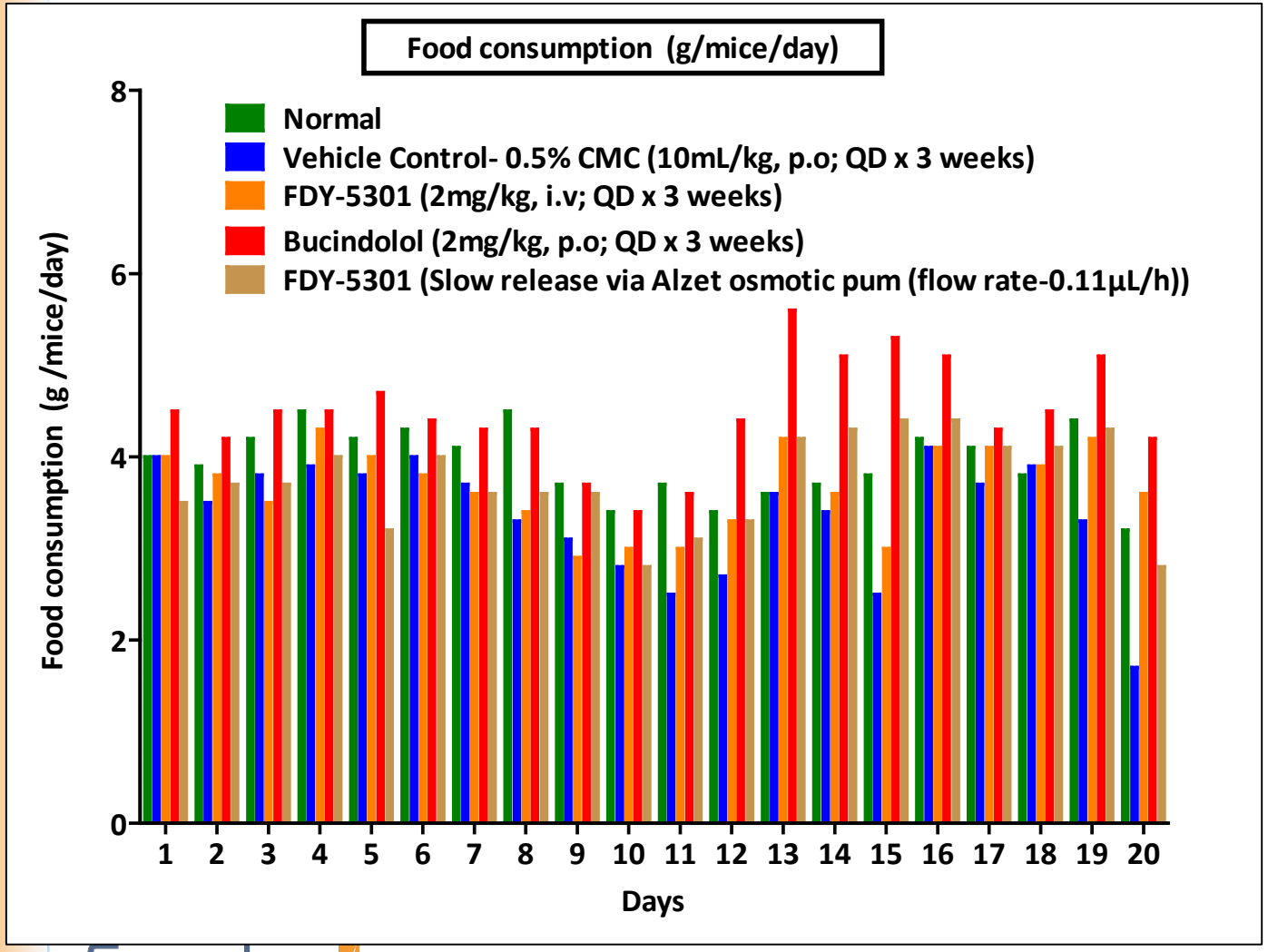
- FDY-5301 and Bucindolol significantly improve body weight over the course of the study.
- Based on ethical reasons and tumor end points, all animals in all experimental groups were humanely euthanized on day 20.

Values are expressed as Mean ± SEM of 10-13 animals in each group.
Based on cage side observations, there were no visible signs of abnormal behavior or clinical symptoms in any of the treated groups

Faraday PHARMACEUTICALS

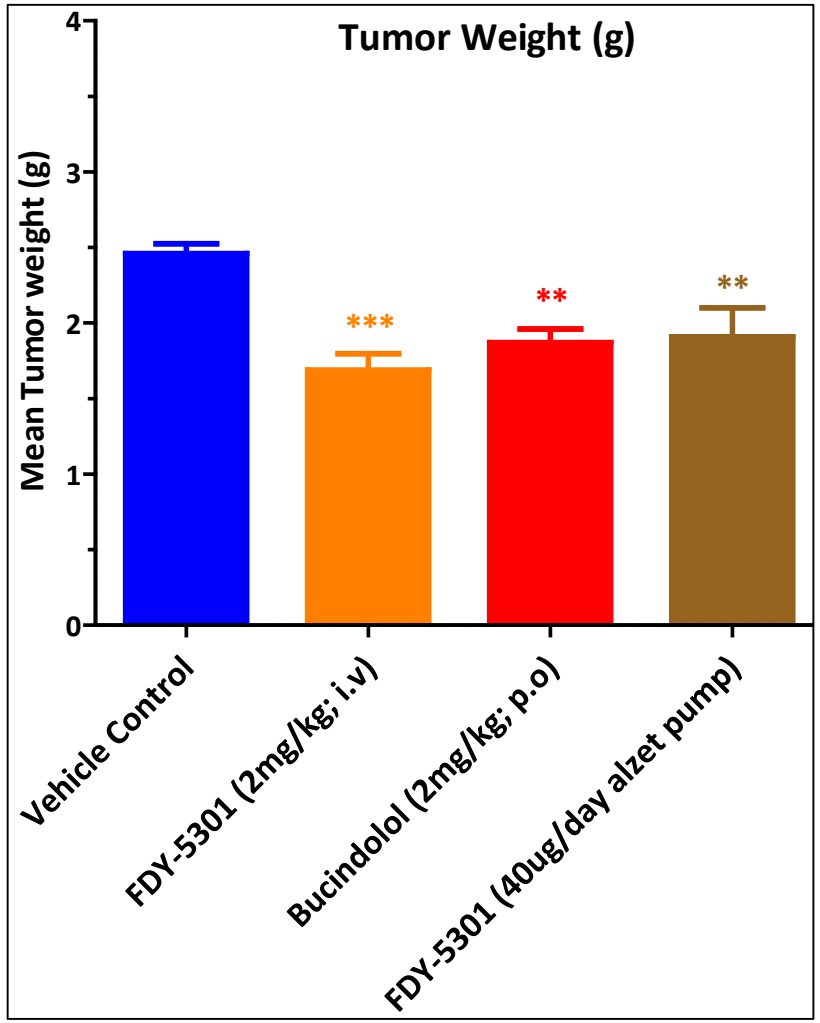
SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Mean feed weight of animals during the study



- The feed consumption pattern was similar in all the groups during the experimental period except vehicle control group, where marginal decrease in feed consumption was observed during the last days of experiment.

Mean Tumor weight



- FDY-5301 (i.v.) significantly decreases tumor weight. $p<0.001$
- FDY-5301 (osmotic pump) & Bucindolol significantly decrease tumor weight, $p<0.01$

Values are expressed as Mean ± SEM of 10 animals in each group.

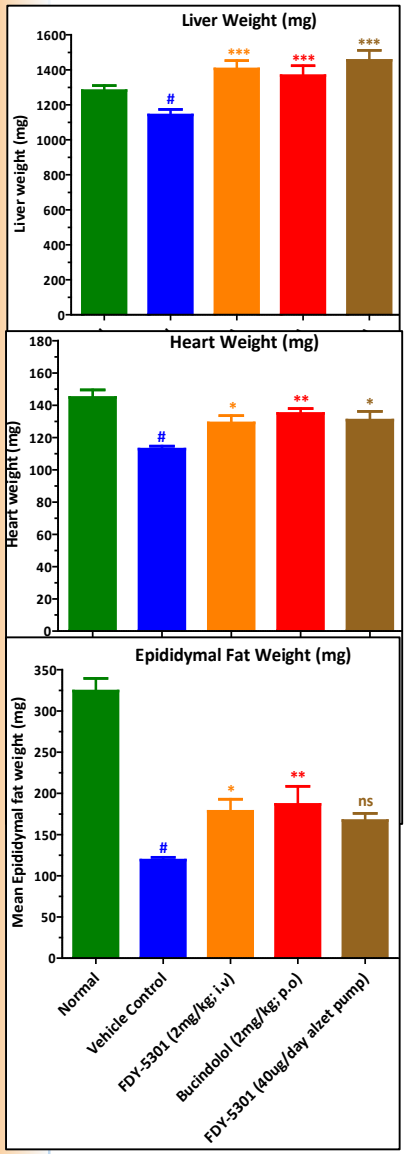
Statistical analysis was carried out by one way ANOVA using Graph Pad Prism (Version.5).

*** $p<0.001$ & ** $p<0.01$ when treatment groups were compared with Vehicle control (Cachexia) group.

Faraday PHARMACEUTICALS

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Average Organ Weight



- FDY-5301 (i.v. & osmotic pump) & Bucindolol preserve body weight, liver and heart weight.
- FDY-5301 (i.v.) & Bucindolol prevent lipolysis (preservation of epididymal fat).

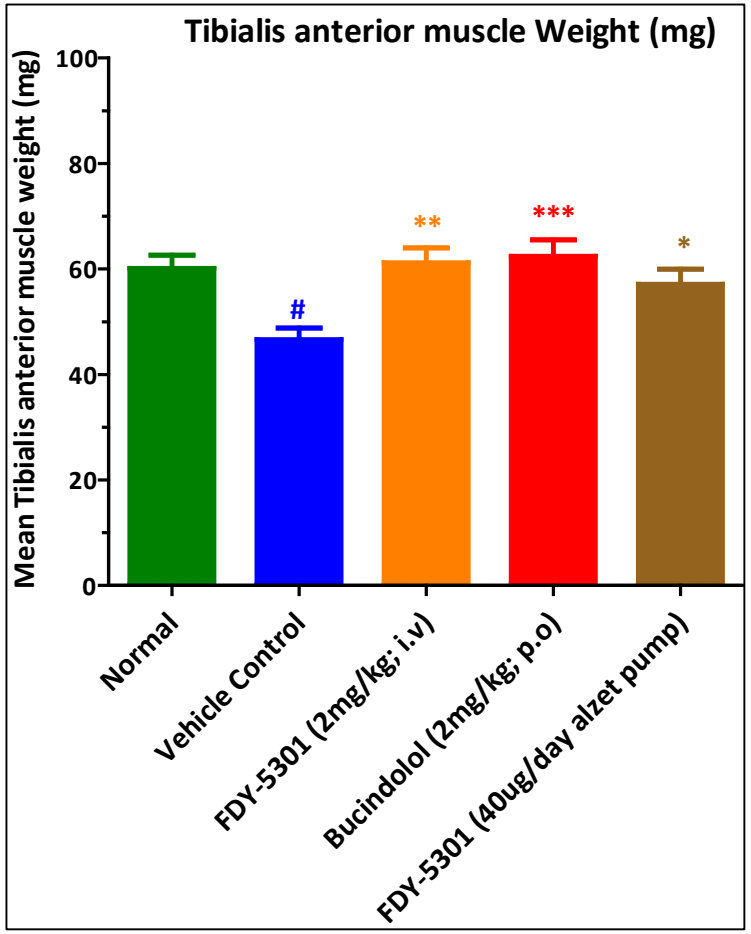
Group	Body Weight on day 20 (g)	Liver (mg)	Heart (mg)	Lung (mg)	Spleen (mg)	Kidney (mg)	Epididymal Fat (mg)
Normal	24 ± 0.5	1282 ± 28	145 ± 5	167 ± 5	111 ± 4	208 ± 10	324 ± 15
Vehicle	19 ± 0.2	1142 ± 31	113 ± 2	152 ± 2	335 ± 24	214 ± 5	119 ± 4
FDY-5301 (i.v.)	**25 ± 0.5*** **	**1406 ± 47*** **	**129 ± 5***	153 ± 4	274 ± 20	196 ± 7	**178 ± 15***
Bucindolol	**25 ± 0.6*** **	**1368 ± 56*** **	**135 ± 3****	165 ± 4	273 ± 24	201 ± 7	**186 ± 22****
FDY-5301 (pump)	**26 ± 0.6*** **	**1454 ± 57*** **	**131 ± 5***	170 ± 8	304 ± 13	206 ± 6	167 ± 9

Values are expressed as Mean ± SEM of 10 animals in each group.
Statistical analysis was carried out by one way ANOVA using Graph Pad Prism (Version.5).
(** p<0.01) when normal (Non-tumor bearing healthy control) was compared with vehicle control group.
*** p<0.001 when treatment groups (FDY-5301 (2mg/kg, i.v), Bucindolol (2mg/kg, p.o), and FDY-5301 (40µg/day; Slow release via Alzet osmotic pump flow rate-0.11µL/h)) were compared with Vehicle control (Cachexia) group.

Faraday PHARMACEUTICALS

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Average Muscle Weight



- FDY-5301 (i.v.) & Bucindolol significantly prevent muscle cachexia, significant preservation of tibialis anterior muscle.

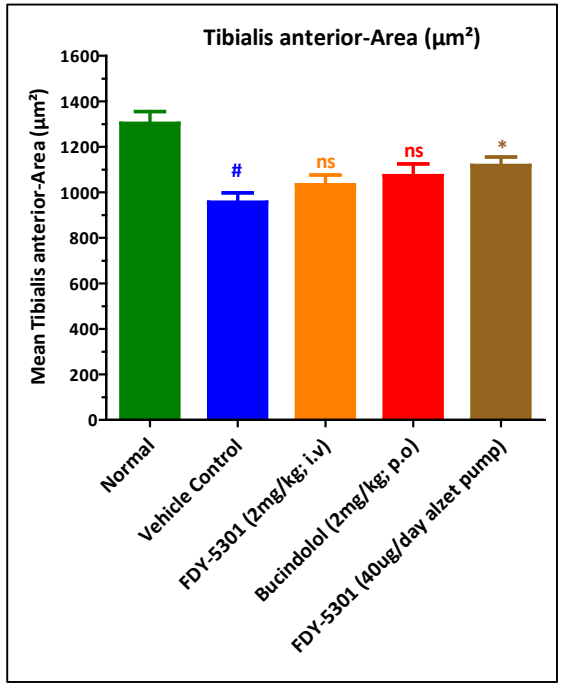
Group	Gastrocnemius (mg)	Tibialis anterior (mg)	Soleus (mg)
Normal	148 ± 11	60 ± 2	7 ± 0.5
Vehicle	107 ± 2	46 ± 2	6 ± 0.3
FDY-5301 (i.v.)	124 ± 4	**61 ± 3****	6 ± 0.3
Bucindolol	116 ± 5	**62 ± 3*****	7 ± 0.3
FDY-5301 (pump)	110 ± 2	57 ± 3*	6 ± 0.2

Values are expressed as Mean ± SEM of 10 animals in each group.
Statistical analysis was carried out by one way ANOVA using Graph Pad Prism (Version.5).
(** p<0.01) when normal (Non-tumor bearing healthy control) was compared with vehicle control group.
*** p<0.001 when treatment groups (FDY-5301 (2mg/kg, i.v), Bucindolol (2mg/kg, p.o), and FDY-5301 (40µg/day; Slow release via Alzet osmotic pump flow rate-0.11µL/h)) were compared with Vehicle control (Cachexia) group.

Faraday PHARMACEUTICALS

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

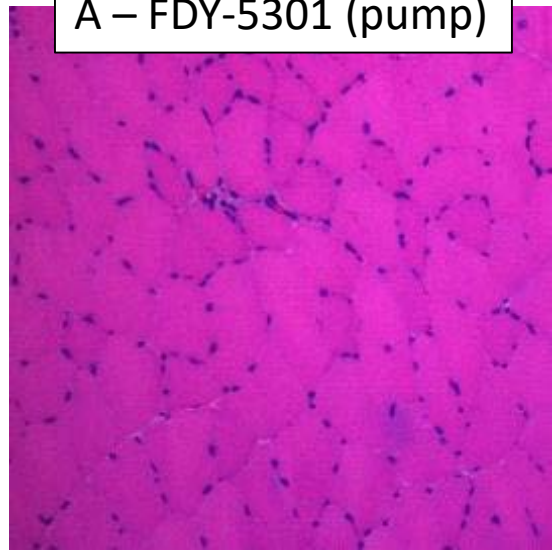
Iodide increases muscle cross sectional area



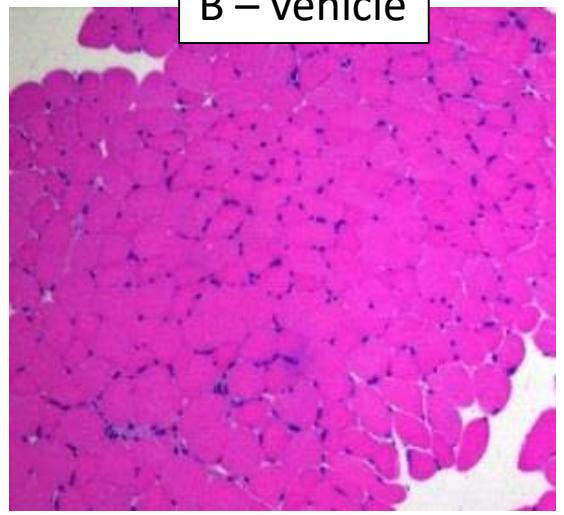
Group	Mean ± SEM
Normal	1303.2 ± 51.4
Vehicle	956.9 ± 40.5
FDY-5301 (i.v.)	1033.5 ± 42.7
Bucindolol	1073.8 ± 51.3
FDY-5301 (pump)	**1118.4 ± 36.7 ***

- Muscle cross sectional area (CSA) is significantly reduced in tumor bearing animals (26.6% reduction).
- Mice treated with 40 µg/day FDY-5301 (delivered by osmotic pump) (A) had a significant increase in tibialis anterior muscle CSA compared to vehicle treatment (B).

A – FDY-5301 (pump)



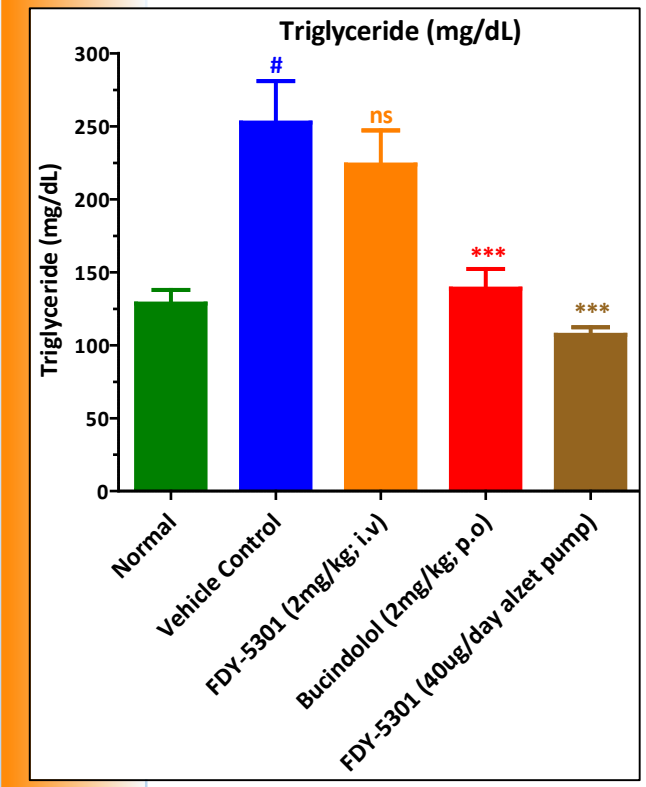
B – vehicle

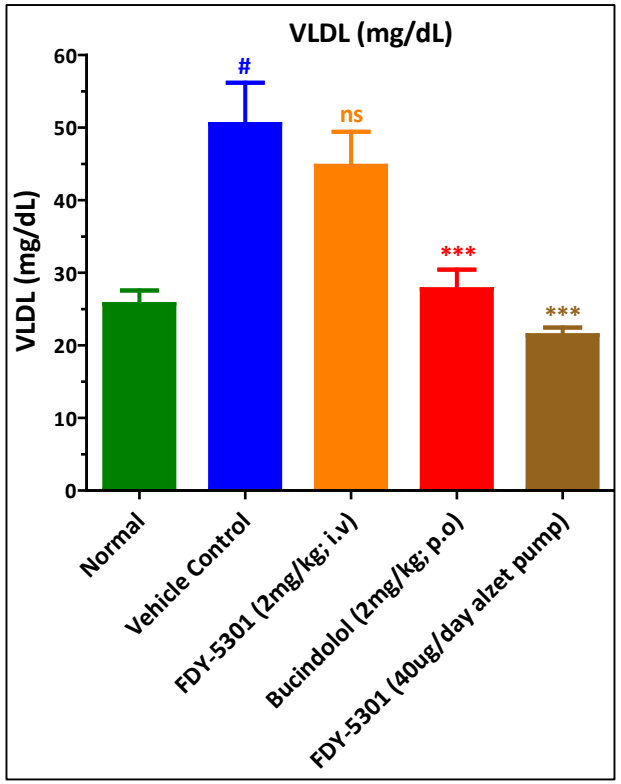


Faraday PHARMACEUTICALS

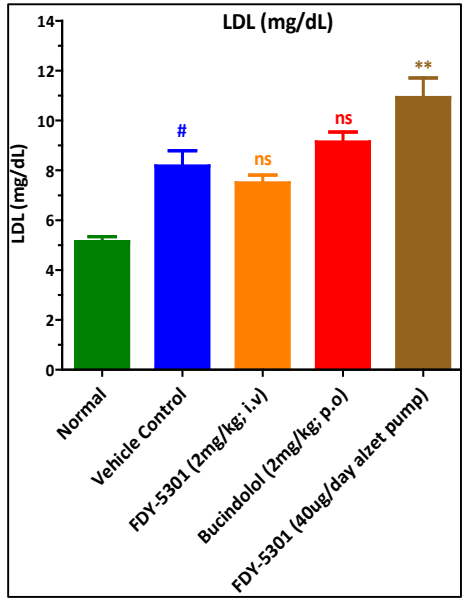
SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Biochemical Analysis of Serum





- FDY-5301 (osmotic pump) & Bucindolol significantly decrease triglycerides and VLDL, while FDY-5301 (osmotic pump) increased LDL.
- No significant change observed in: cholesterol, HDL, glucose, or total protein

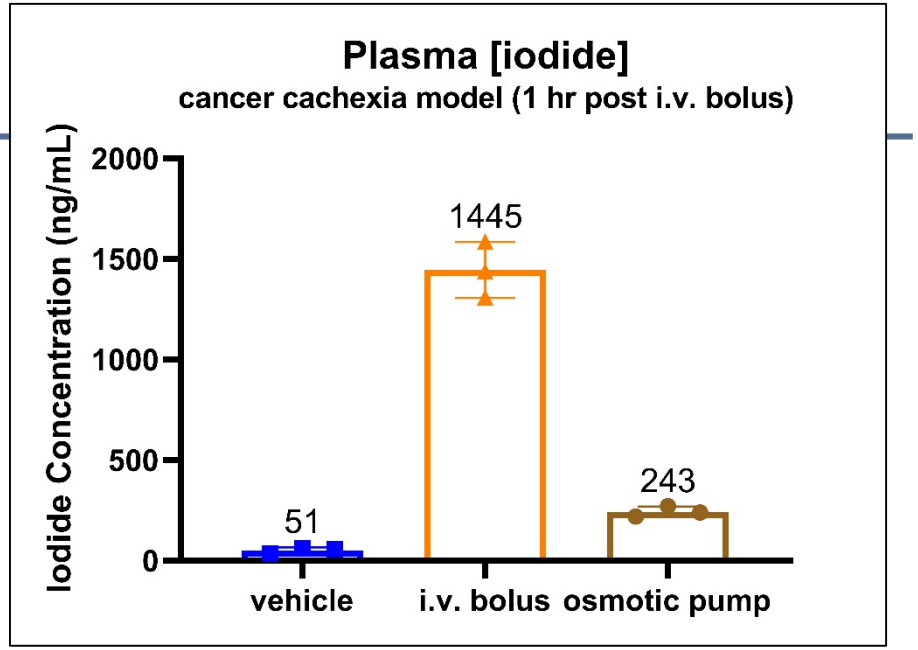


Faraday PHARMACEUTICALS

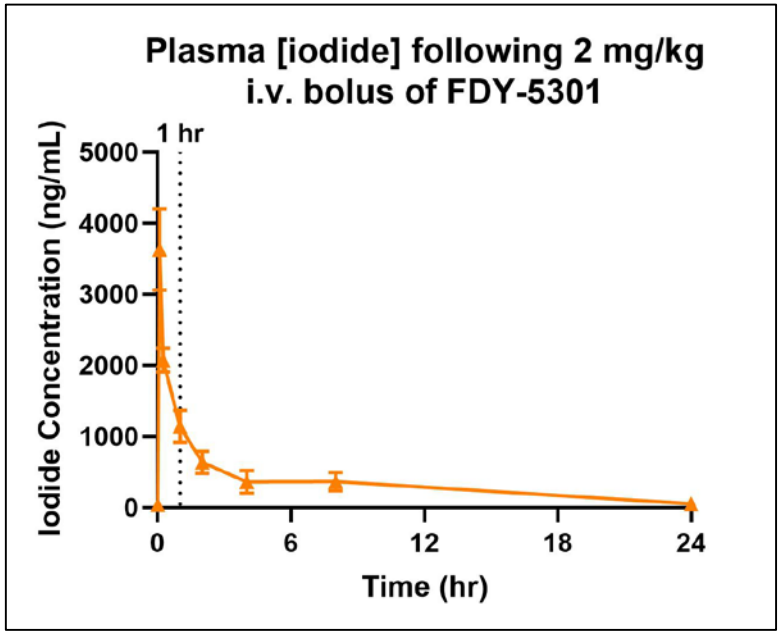
SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

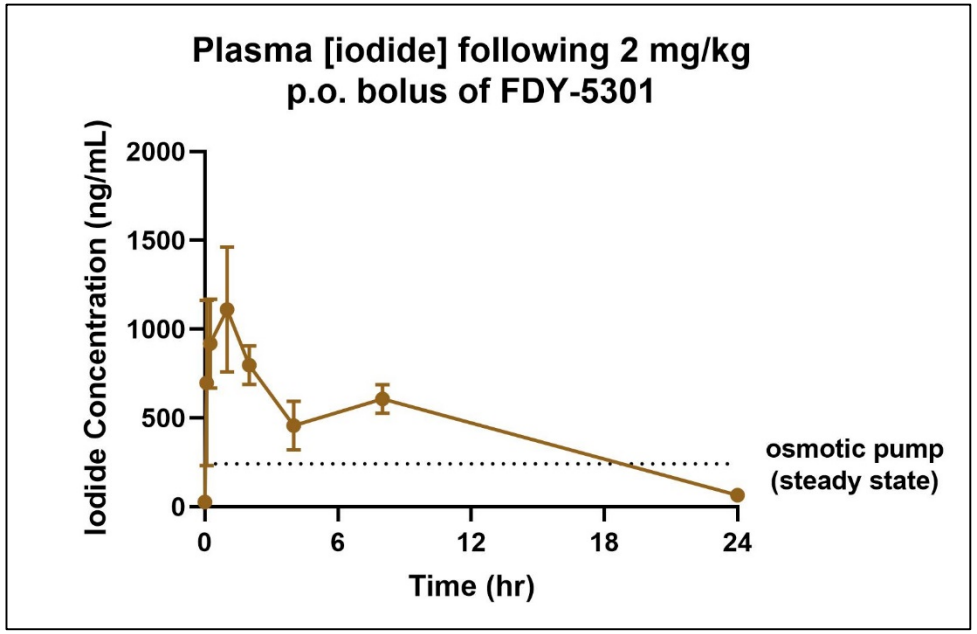
Plasma [iodide]

- Plasma iodide concentration is ~5x to ~30x higher than endogenous iodide levels following osmotic pump or i.v. bolus administration, respectively).
- Iodide was assessed using Ion Chromatography with amperometric detection.



- Plasma [iodide] time curve following i.v. or p.o. bolus (naïve mice, no tumor)





Faraday PHARMACEUTICALS

SCWD SOCIETY ON SARCOPENIA, CACHEXIA AND WASTING DISORDERS

Acknowledgements

This study was run at Syngene International Ltd, (Bangalore, India) and I would like to thank:

- Balaji Ramachandran
- Aravindakshan Jayaprakash
- Rajendiran Satheesh
- Venkidusamy Rajendran

for their contributions on this project.

Conclusions

FDY-5301 (iodide)

- reduces inflammation
- reduce oxidative stress
- catalytically disproportionate hydrogen peroxide

In this model we demonstrate that iodide:

- slows tumor growth progression
- preserves body weight & organ weight
- prevents muscle cachexia
- Increases muscle CSA