Investor Relations 2019

ENZYCHEM LIFESCIENCES

Global New Drug Development Company

Investor Relations 2019

2019. 10

ENZYCHEM LIFESCIENCES

SAFE HARBOR STATEMENTS

THIS PRESENTATION AND THE INFORMATION IN IT ARE PROVIDED IN CONFIDENCE, FOR THE SOLE PURPOSE OF EXPLORING BUSINESS OPPORTUNITIES BETWEEN ENZYCHEM LIFESCIENCES AND THE RECEIVING PARTY AND MAY NOT BE DISCLOSED TO ANY THIRD PARTY OR USED FOR ANY OTHER PURPOSE WITHOUT THE EXPRESS WRITTEN PERMISSION OF ENZYCHEM LIFESCIENCES.

ENZYCHEM LIFESCIENCES AND ITS EMPLOYEES WILL NOT BE LIABLE FOR ANY SPECIAL, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES OF ANY KIND, INCLUDING, WITHOUT LIMITATION, ANY LOSS OF PROFIT, LOSS OF USE, OR BUSINESS INTERRUPTION, BASED ON ANY CLAIMS UNDER THIS PRESENTATION.

THIS PRESENTATION AND THE INFORMATION IN IT MAY CONTAIN FORWARD-LOOKING STATEMENTS, THE ACHIEVEMENT OR SUCCESS OF WHICH INVOLVES RISKS, UNCERTAINTIES, AND ASSUMPTIONS. THE RISKS AND UNCERTAINTIES REFERRED TO INCLUDE – BUT ARE NOT LIMITED TO – RISKS AND UNCERTAINTIES ASSOCIATED WITH THE GENERAL ECONOMIC AND MARKET CONDITIONS, PERFORMANCE OF THE COMPANY, AND FUTURE CLINICAL STUDIES WITHOUT THIRD PARTY VERIFICATION. THE PREDICTION CONTAINS UNCERTAINTY IN NATURE AND THE ACTUAL FUTURE EVENT MAY VARY FROM THE PREDICTION PRODUCED IN THIS PRESENTATION.

Healthy and Happy Life

Enzychem Lifesciences & EC-18

ENZYCHEM LIFESCIENCES

ENZYCHEM LIFESCIENCES: Company Overview

COMPANY HIGHLIGHTS

- Founded in 1999
- KOSDAQ:183490 (Listed in Feb 2018)
- Market cap: USD 764M (as of end of 2018)
- More than 100 employees in United States and South Korea
- Two GMP Manufacturing Facilities in Jecheon, South Korea

- Presentations on NASH selected by 2019 AASLD and on anti-metastasis and synergistic effect on anti-tumor selected by 2019 AACR-NCI-EORTC molecular targets conference
- Ongoing two phase 2 clinical trials for chemoradiation-induced oral mucositis (CRIOM) and chemotherapy-induced neutropenia (CIN) and in animal rule study *in lieu* of phase 2 in the US for acute radiation syndrome (ARS)
- EC-18 was selected to collaborate with the US Government; the Radiation and Nuclear Countermeasures Program (RNCP) and Chemical Countermeasures Research Program (CCRP) by the National Institute of Allergy and Infectious Diseases (NIAID) and the Armed Forces Radiobiology Research Institute (AFRRI) by Department of Defense (DoD).
- EC-18 received the Fast Track Designation for CRIOM and Orphan Drug Designation for ARS by US FDA

Three Operating Business Segments

ENZYCHEM LIFESCIENCES

New Drug Development

Proprietary EC-18 Platform Technology

- CIN, CRIOM, ARS
- NASH/Diabetes mellitus
- Cancer metastasis
- Inflammatory disease (RA, sepsis, psoriasis)

More than $100B
GLOBAL MARKET POTENTIAL

API

Stable Revenue Generating Business

- Analgesics
- Anti-coagulants
- Expectorants
- Anti-tuberculosis

$200M MARKET POTENTIAL

Contrast Agents

Contrast Imaging Products

- MRI contrast agents
- Low risk generic MRI contrast agents

$450M MARKET POTENTIAL

DIVERSIFIED REVENUE BUSINESSES TO SUPPORT INVESTMENTS IN PHARMACEUTICALS

Leadership Team

Ki Young Sohn
Chairman & CEO
- Over 30 years of experience in the pharmaceutical and finance industry
- Chairman of Bridget Lifescience; Former Director at Samil PwC
- Author of 11 EC-18 scientific papers

Hye Kyung Kim
Vice Chairman
- Over 30 years of experience in health functional food
- CEO, Bridget Lifescience
- Former Vice Chair, KONEX Conference

Myung Hwan Kim, MD, PhD
Chief Medical Officer
- Director, Center for Pancreatobiliary Diseases, AMC
- Professor, Division of Gastroenterology, University of Ulsan College of Medicine
- President, Asian-Oceanic Pancreatic Association

Jae Yong Lee
Vice President
- Over 30 years of experience in API industry and manufacturing
- Former Director at Offi-Com and Adtech
- Former CEO, Hanseung C&S

DoHyun Cho, PhD
Chief Operating Officer
- Over 20 years of experience in healthcare
- Head of KHDI USA
- CEO, W Medical Strategy Group

Do Young Lee, PhD
Chief Scientific Officer
- 23 years of experience in New Drug Development
- Former Head of Translational Research, CrystalGenomics
- 2 successful NDA filings in Korea

Changgi D. Hong, MD
Inventor of EC-18
- Former President, Asan Medical Center
- Former President, Asan Healthcare System
- Former Professor of Medicine, University of Cincinnati

Scientific Advisory Board

ENZYCHEM LIFESCIENCES

Jeff Crawford, MD (Chairman)

- Professor, Duke University School of Medicine
- Chair, NCCN Myeloid Growth Factor Committee
- Lead Investigator of Clinical Trials for Neupogen & Neulasta

Stephen Sonis, DMD, DMSc

- Professor, Harvard School of Dental Medicine
- Senior Surgeon, the Dana-Farber Cancer Institute and Brigham and Women's Hospital
- In Chage of U.S. Phase II Clinical Trial of Oral Mucostis

David Grdina, PhD

- Professor, University of Chicago
- Member of Reviewers Reserve, NIH
- Ad Hoc Member of Special Emphasis Panel, NCI

Ronald Manning, PhD

- Over 10 years of specialized experience in MCM on ARS with BARDA, NIH, FDA and DOD
- Former Branch Chief, BARDA
- Former Professor, Vanderbilt Univ. School of Medicine

Jae Wha Kim, PhD

- Professor, University of Science and Technology (UST)
- Senior Researcher, KRIBB
- Postdoctoral Fellow in Molecular Genetics & Microbiology, College of Medicine Univ. of Florida

Larry Kwak, MD

- Vice President, City of Hope Cancer Center
- Director, Toni Stephenson Lymphoma Center
- Named one of TIME magazine's "100 Most Influential People" in 2010

Kyu Pyo Kim, MD, PhD

- Professor, Dept. Medical Oncology, Asan Medical Center
- Visiting Scholar, Cancer PK and PD Core Cancer Therapeutics Program, University of Pittsburgh
- Researcher, KHIDI Ministry of Health Korea

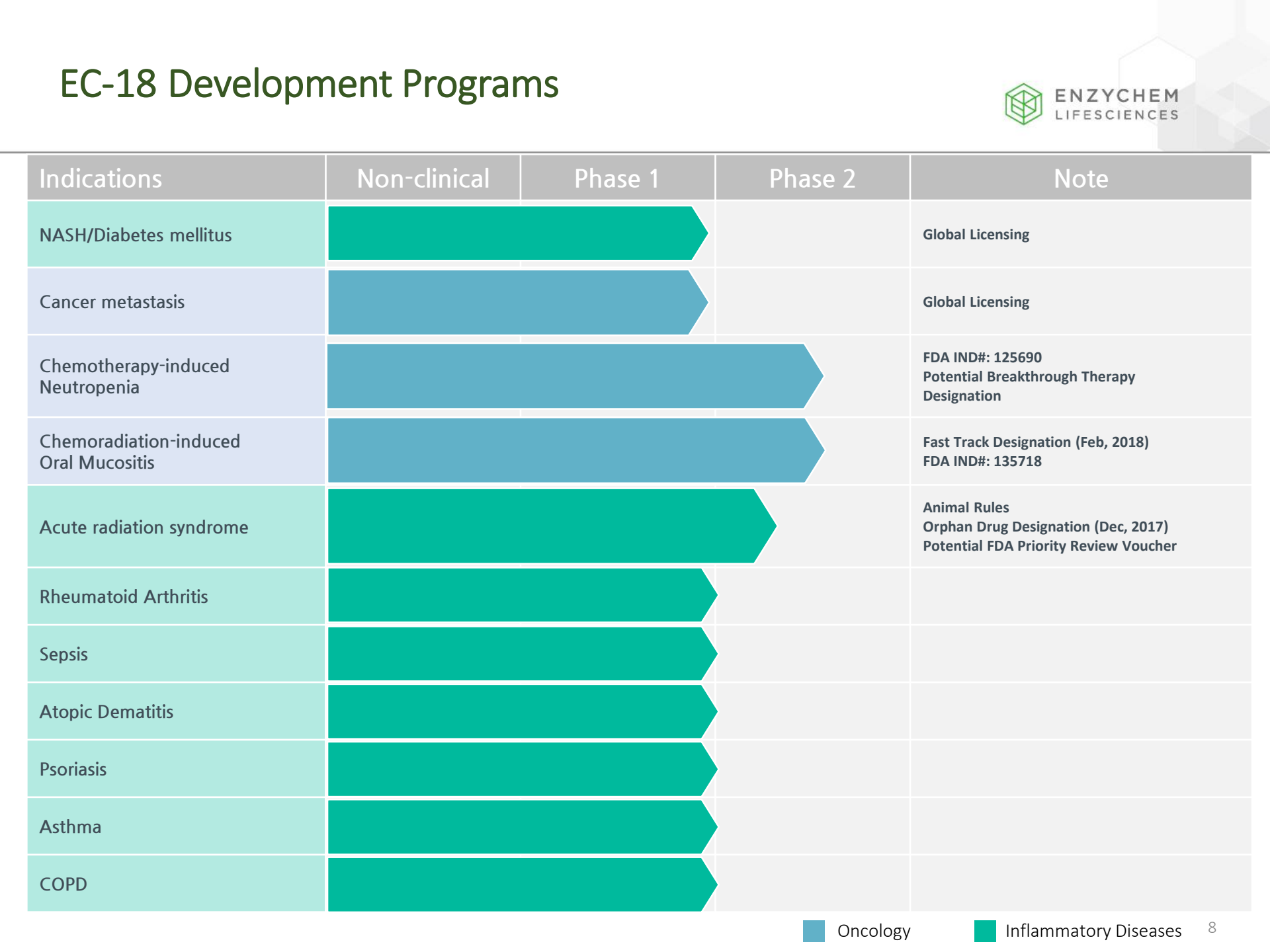
Soon Kil Ahn, PhD

- Dean & Professor, Incheon National University
- Director, Insitutute for New Drug Development, Incheon National University
- Former Executive Director, Chong Kun Dang Pharm

EC-18 Development Programs

ENZYCHEM LIFESCIENCES

Indications	Non-clinical / Phase 1 / Phase 2	Note
NASH/Diabetes mellitus		Global Licensing
Cancer metastasis		Global Licensing
Chemotherapy-induced Neutropenia		FDA IND#: 125690 Potential Breakthrough Therapy Designation
Chemoradiation-induced Oral Mucositis		Fast Track Designation (Feb, 2018) FDA IND#: 135718
Acute radiation syndrome		Animal Rules Orphan Drug Designation (Dec, 2017) Potential FDA Priority Review Voucher
Rheumatoid Arthritis		
Sepsis		
Atopic Dematitis		
Psoriasis		
Asthma		
COPD		



EC-18 Immune Modulator Platform Compound

ENZYCHEM LIFESCIENCES

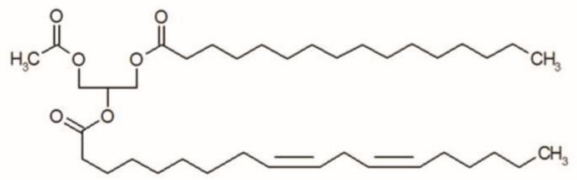
A REVOLUTIONARY COMPOUND ACTIVE IN MULTIPLE DISEASES

An innovative medicine for oncology and inflammatory disease

NOVEL TECHNOLOGY WITH OVER 40+ YEARS RESEARCH

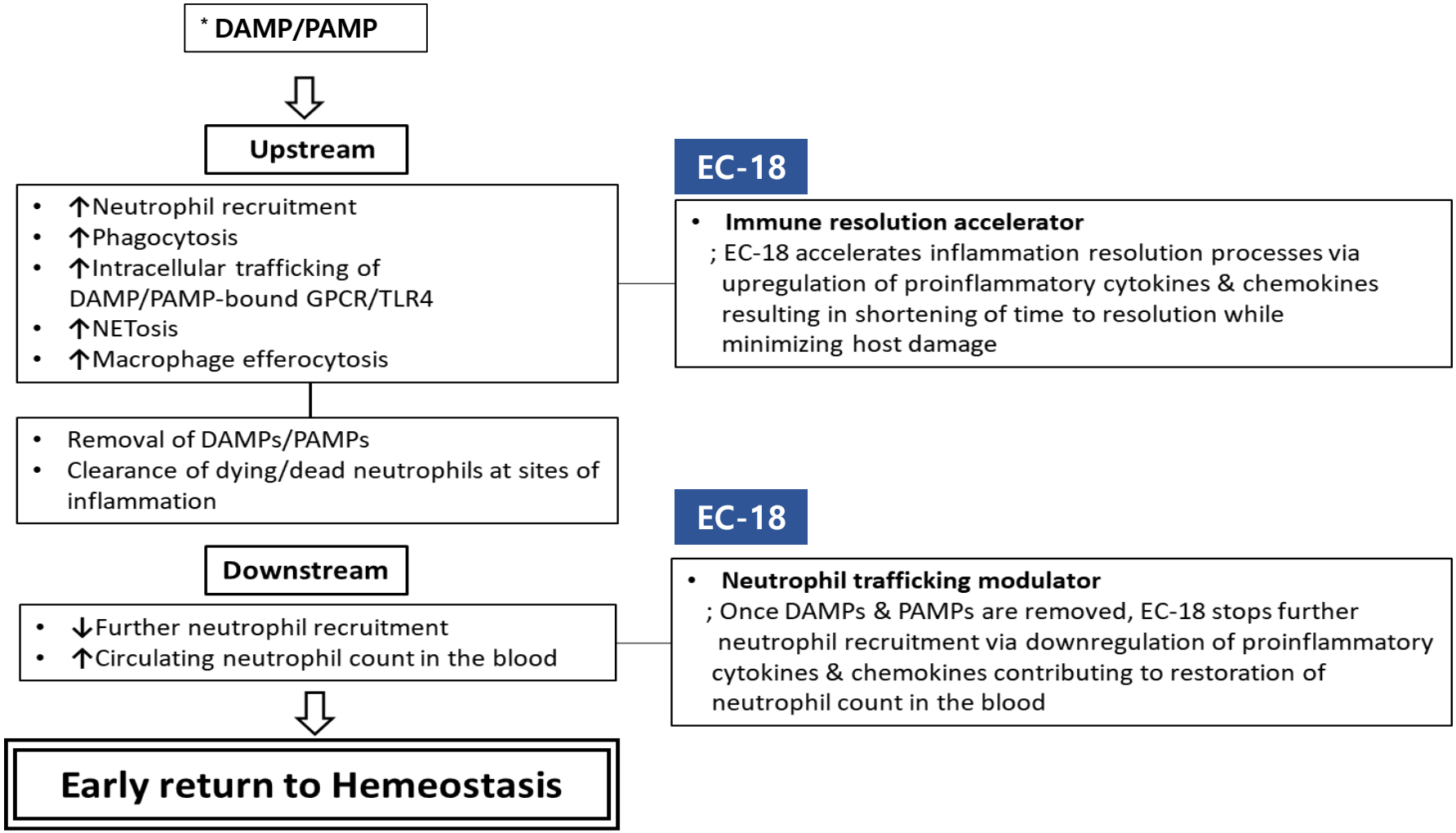
EC-18 is a safe, orally available, lipid-based, first-in-class, small molecule drug

- EC-18 is a synthetic 1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol (PLAG)
- Immune resolution accelerator (IRA) and neutrophil trafficking modulator (NTM)
- Global Phase 2 clinical trials in CIN, CRIOM and ARS (animal rule study *in lieu* of phase 2)
- FDA Fast Track Designation in CRIOM
- FDA Orphan Drug Designation in Acute Radiation Syndrome (ARS)



EC-18

MOA of EC-18



- DAMP: Damage-associated molecular pattern
  PAMP: Pathogen-associated molecular pattern

AACR Annual Meeting 2019, Atlanta

ENZYCHEM LIFESCIENCES

Poster No. 4586

ENZYCHEM LIFESCIENCES

KRIBB Korea Research Institute of Bioscience & Biotechnology

PLAG enhances macrophage mobility for efferocytosis of active neutrophils via membrane re-distribution of P2Y2

GUEN TAE KIM[1], DO YOUNG LEE[2], KI-YOUNG SOHN[2], SUN YOUNG YOON[2], JAE WHA KIM[1]

[1] Korea Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Republic of Korea

[2] ENZYCHEM lifescience, Jecheon-si, Republic of Korea

Abstract

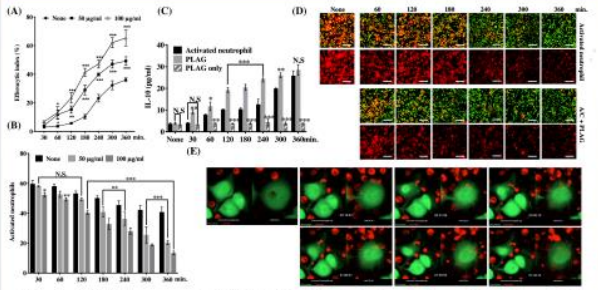
Neutrophil activity is prerequisite during chemotherapy. The DAMP (Damage Associated Molecular Pattern) molecules generated by chemotherapy could be effectively trapped by activated neutrophil called 'NETosis'. Efferocytosis of macrophages should remove most activated neutrophils including NETosis A timely removal of activated neutrophils is essential for the prevention of abnormal activation of immune response and metastatic activity of cancer cells induced by tumor microenvironment (TME). Particularly, appropriate clearance of the activated neutrophils by efferocytosis should be carried out because activated neutrophils have a detrimental effect on TME.

In this research, we investigated the effect of 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) on efferocytosis and its underlying molecular mechanisms. In a co-culture of activated neutrophils with macrophages, PLAG increased the activity of efferocytosis for elimination of activated neutrophils. PLAG accelerated translocation of P2Y2 from lipid rafts to non-lipid-raft plasma membrane domains in macrophages. This repositioning of P2Y2 enables the polarization of the cytoskeleton by association of the receptor with cytoskeletal proteins such as α-tubulin and actin to improve the mobility of macrophages. Through these protein assemble, PLAG encouraged macrophage mobility toward the activated neutrophils. Formation of micelle including PLAG, chylomicron-like structures, was a prerequisite for induction of this macrophage activity. PLAG effect on this activity was not observed in the absence of GPIHBP1,micelle receptor.

Taken together, these data showed that PLAG triggered a prompt clearance of activated neutrophils through enhancement of efferocytosis activity. Subsequently, PLAG could have effects on modulation of TME. PLAG could be utilized as an effective lipid-based TME modulator via the prevention of abnormal activation induced by uncontrolled immune response during chemotherapy.

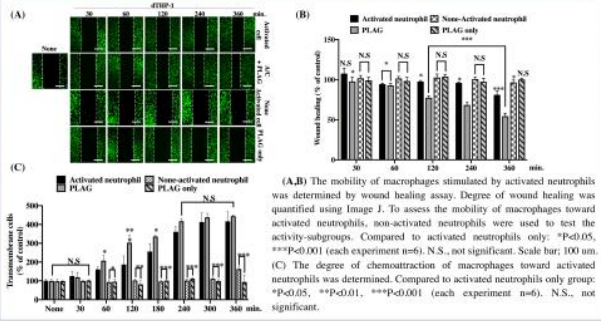
Result

Effect of PLAG on the induction of activated neutrophil efferocytosis



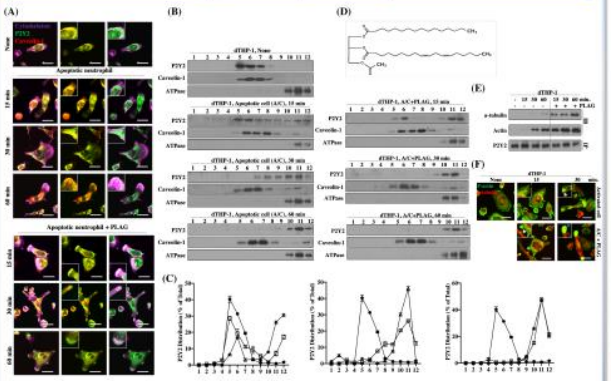
Differentiated THP-1 cells were pre-treated with PLAG for 1 h and then stimulated by activated neutrophils. **(A)** Efferocytotic index was calculated by FACS. Compared to activated neutrophil only group: *P<0.05, **P<0.01, ***P<0.001 (each experiment n=6). **(B)** Number of un-engulfed activated neutrophils was quantitated by FACS. Compared to control: *P<0.05, **P<0.01, ***P<0.001 (each experiment n=6). **(C)** Changes in IL-10 cytokine levels in the culture medium were determined by ELISA. Compared to control: *P<0.05, **P<0.01, ***P<0.001 (each experiment n=6). N.S., not significant. **(D)** The degree of clearance of apoptotic neutrophils was confirmed using confocal microscopy. Activated neutrophils were tagged with red fluorescence and macrophages tagged in green fluorescence. Scale bar; 100 um. **(E)** Efferocytosis of macrophages was visualized in real time.

Increase of macrophage mobility on the PLAG treated cells



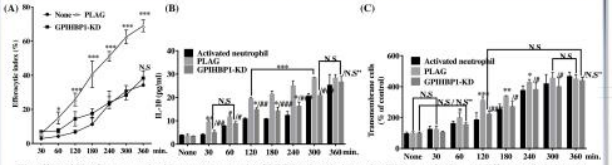
(A,B) The mobility of macrophages stimulated by activated neutrophils was determined by wound healing assay. Degree of wound healing was quantified using Image J. To assess the mobility of macrophages toward activated neutrophils, non-activated neutrophils were used to test the activity-subgroups. Compared to activated neutrophils only: *P<0.05, ***P<0.001 (each experiment n=6). N.S., not significant. Scale bar; 100 um. **(C)** The degree of chemoattraction of macrophages toward activated neutrophils was determined. Compared to activated neutrophils only group: *P<0.05, **P<0.01, ***P<0.001 (each experiment n=6). N.S., not significant.

Enhanced movement of P2Y2 receptor from the lipid raft to non-lipid raft in the PLAG treated cells

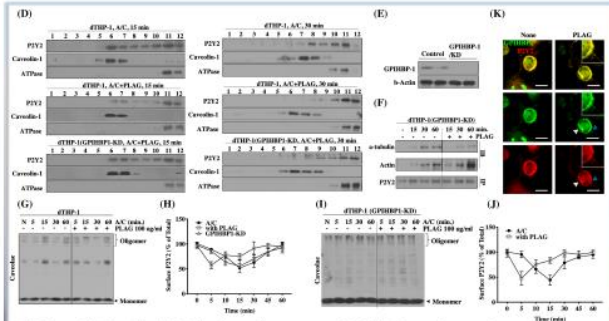


(A) Co-localization shift of P2Y2 and co-binding with cytoskeleton by membrane distribution change were confirmed by confocal. White arrow indicated that P2Y2 were co-localization with caveolin-1 and blue arrow indicate that P2Y2 were co-localization with cytoskeleton. Scale bar; 20 um. **(B)** The membrane distribution change of P2Y2 was determined by the lipid raft fractionation method. Caveolin-1 was used as a lipid raft marker. **(C)** The distribution of P2Y2 in each band was quantified and plotted. ●: None, □:Apoptotic neutrophil, △: PLAG. **(D)** The simple structure of PLAG. **(E)** The binding of P2Y2 with proteins related to polarization of the cytoskeleton was detected by immunoprecipitation. **(F)** The degree of cytoskeletal polarization and colocalization with actin protein was determined by confocal microscopy. Scale bar; 20 um

Promoted movement of P2Y2 receptor to non-lipid raft by structural PLAG was dependent on GPIHBP1, vesicle recognizing receptor

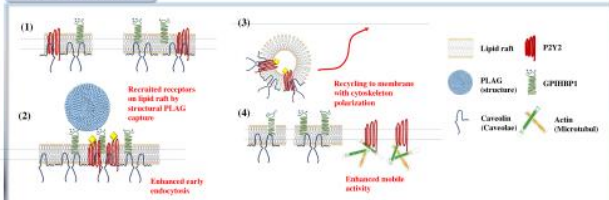


The effect of PLAG was examined in macrophages with GPIHBP-1 knock-down via siRNA transfection. **(A)** Efferocytotic index was calculated by FACS. Compared to activated neutrophil only group: *P<0.05, ***P<0.001 (each experiment n=6). N.S., not significant.



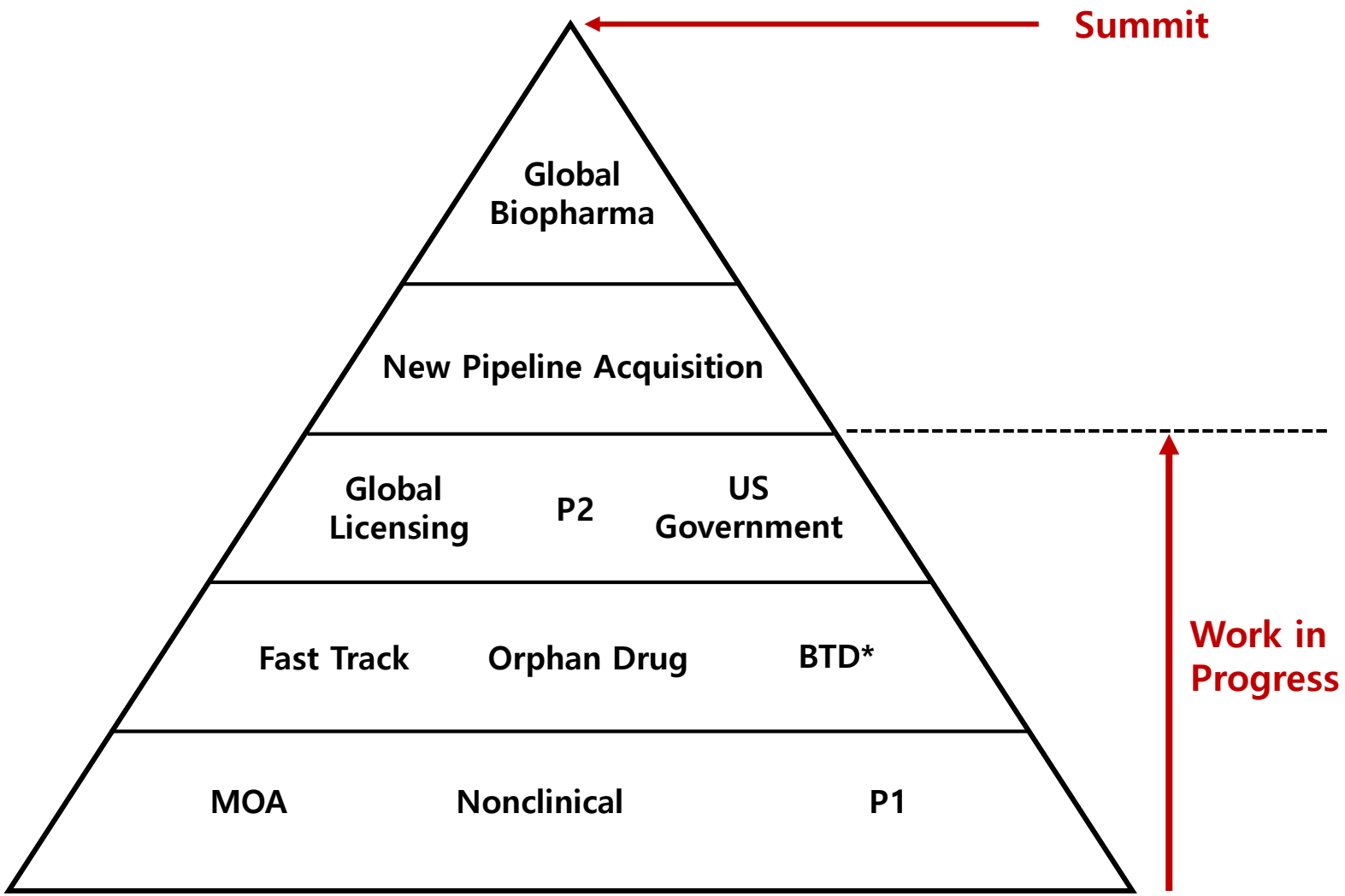
(B) Changes of IL-10 cytokine levels in the culture medium were measured by ELISA. Compared to apoptotic neutrophil only group: *P<0.05, **P<0.01, ***P<0.001 (each experiment n=6). Compared to PLAG group: #P<0.05, ##P<0.01, ###P<0.001 (each experiment n=6) N.S., not significant (Compared to apoptotic neutrophil only group). N.S.", not significant (Compared to PLAG group). **(C)** The degree of chemoattraction of macrophages toward apoptotic neutrophils was determined. Compared to apoptotic neutrophil only group: *P<0.05, **P<0.01, ***P<0.001 (each experiment n=6). Compared to PLAG group: #P<0.05, ##P<0.01, ###P<0.001 (each experiment n=6) N.S., not significant (Compared to apoptotic neutrophil only group). N.S.", not significant (Compared to PLAG group). **(D)** The membrane distribution change of P2Y2 in GPIHBP-1 knock-down cells was confirmed by the lipid raft fraction method. Change of P2Y2 distribution by PLAG treatment was quantified at the same time. **(E)** Knock-down of GPIHBP-1 via siRNA transfection was confirmed. **(F)** Co-immunoprecipitation of P2Y2 with proteins related to polarization of the cytoskeleton. **(G,I)** The changes of caveolae formation in Lipid raft over time were confirmed by Western blotting. **(H,J)** The surface membrane expression of P2Y2 (Trafficking) over time was quantitated using FACS. **(K)** The co-localization and polarization changes of GPIHBP1 and P2Y2 by structural PLAG treatment were confirmed by Confocal Scale bar; 20 um.

Conclusion



Our study shows that the action of structural PLAG induces recruitment of GPIHBP1, which is GPI-AP, and caveolae that formed through it rapidly accelerated the trafficking activity of P2Y2 for cytoskeleton rearrangement. The promotion of P2Y2 receptor translocation by PLAG provides more rapid movement of macrophages toward nucleotides generated by activated neutrophils, allowing the macrophages to rapidly eliminate the activated neutrophils.

Road to Global Biopharma



* BTD : Breakthrough Therapy Designation

Healthy and Happy Life

Global Licensing

ENZYCHEM LIFESCIENCES

Healthy
and
Happy Life

Nonalcoholic Steatohepatitis (NASH)

2019 AASLD Selected Abstract Information

EC-18, A NOVEL IMMUNE RESOLUTION ACCELERATOR, IMPROVES NASH AND LIVER FIBROSIS

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease with significant unmet need. NAFLD encompasses a spectrum of fatty liver disease, ranging from steatosis to nonalcoholic steatohepatitis (NASH) accompanied by hepatocyte ballooning and inflammation and fibrosis. To identify potential new drug for NASH and fibrosis, we investigated whether EC-18 (1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol, PLAG) shows improvement in both NASH and fibrosis via accelerating resolution of inflammation.

Methods: To investigate the efficacy of EC-18 on liver steatosis induced by acute streptozotocin (STZ)-induced β cell damage, STZ 200 mg/kg was intraperitoneally injected to 10-week old Balb/c mice at Day 1 and vehicle and EC-18 were daily administered for 3 days. And to evaluate its efficacy on NASH and fibrosis, we tested EC-18 in a STAMTM mouse model (SMC Lab. Tokyo). Mice were s.c. injected with STZ 200 mg at day after birth and high fat diet for 6 weeks starting at 4 weeks of age. EC-18, OCA (obeticholic acid), MGL-3196 and EC-18 were orally administered for 3 weeks, starting at 6 weeks of age. To ameliorating effect of EC-18 on inflammation and lipotoxicity in hepatocytes under insulin resistance, we used high-fat-high-fructose (HFHF)-dieted ICR mice.

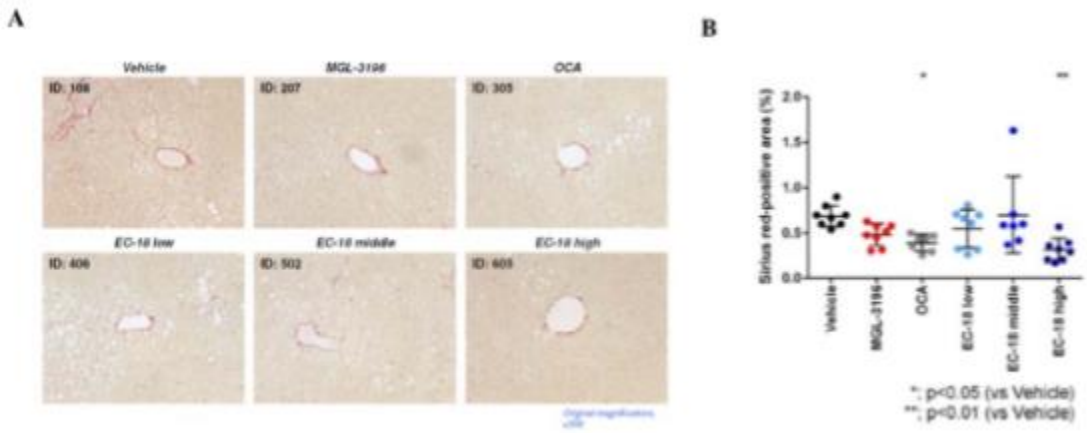
Results: In acute STZ-induced hepatic steatosis model, EC-18 significantly improved STZ-induced histological hepatic steatosis as confirmed by Oil red O and H&E staining. Blood and liver levels of triglyceride (TG) were decreased following EC-18 treatment (70.7% and 31.3%, respectively, $p<0.05$). EC-18 restored lipoprotein lipase expression levels in muscle, which were distinctly reduced by STZ treatment, and resulted in the improvement of muscle atrophy and muscle function. In STAMTM NASH & fibrosis model, EC-18 significantly reduced NASH disease activity based on key histological parameters including steatosis & hepatocyte ballooning ($p<0.05$) compared to the vehicle-treated group. The percentage of fibrosis area (Sirius res-positive area) significantly decreased in the EC-18 treatment group relative to the vehicle, with mice dosed at the highest concentration (250mg/kg) ($p<0.01$). Notably, the reduction of fibrosis through Sirius-red staining was more significant than that of OCA and MGL-3196. In HFHF-induced insulin resistance mice model, EC-18 attenuated hepatocyte inflammation and cell damage induced by feeding with HFHF. EC-18 reduced the secretion of HMGB1 and LDH from the damaged hepatocytes and attenuated the phosphorylation of RIPK1.

Conclusion: In various mice models, EC-18 ameliorated hepatic steatosis, NASH and liver fibrosis via regulating lipid metabolism in peripheral tissue and attenuating hepatocyte inflammation and cell damage caused by damage-associated molecular patterns (DAMPs). In conclusion, EC-18 can be a promising therapeutics to resolve NASH and to prevent progression to liver fibrosis.

2019 AASLD Selected Abstract Information

EC-18, A NOVEL IMMUNE RESOLUTION ACCELERATOR, IMPROVES NASH AND LIVER FIBROSIS

Conclusion: In various mice models, EC-18 ameliorated hepatic steatosis, NASH and liver fibrosis via regulating lipid metabolism in peripheral tissue and attenuating hepatocyte inflammation and cell damage caused by damage-associated molecular patterns (DAMPs). In conclusion, EC-18 can be a promising therapeutics to resolve NASH and to prevent progression to liver fibrosis.



Summary

ENZYCHEM LIFESCIENCES

1. EC-18 significantly reduced NASH disease activity score based on key histological parameters including steatosis & hepatocyte ballooning.

2. EC-18 also significantly reduced liver fibrosis, plasma CK-18 fragments & inflammation area assessed by F4/80 stain as compared with vehicle control & reference compounds.

3. These results are highly relevant because the primary endpoint of clinical trial is resolving NASH and reducing progression to fibrosis.

4. EC-18 was superior to reference compounds (OCA, MGL3196, CVC) undergoing phase 3 clinical trial treating NASH.

Conclusion

In various mice models, EC-18 as an immune resolution accelerator ameliorated hepatic steatosis, NASH and liver fibrosis via enhancing lipid metabolism in peripheral tissue and attenuating hepatocyte inflammation and cell damage caused by damage-associated molecular patterns (DAMPs). In conclusion, EC-18 can be a promising therapeutics to resolve NASH and to prevent progression to liver fibrosis.

Advantages of EC-18 in NASH & Fibrosis

ENZYCHEM LIFESCIENCES

1. Excellent drug safety based on preclinical & clinical data

2. No need of conducting phase 1 clinical trial for drug development (already completed)

3. Efficacy in both NASH (nonalcoholic steatohepatitis) and hepatic fibrosis

; Although fibrosis is not a requirement for the diagnosis of NASH, fibrosis is often present in NASH patients. NASH without fibrosis , if untreated , can progress to advanced fibrosis (NASH with fibrosis). Most clinical trials in NASH have indicated improved NASH with no worsening (but not improvement) of hepatic fibrosis or improved hepatic fibrosis with no worsening of NASH. Few compounds both reduce disease activity (NASH) and also prevent progression to hepatic fibrosis. EC-18 is effective in both ameliorating disease activity of NASH and reducing progression to fibrosis.

4. EC 18 has additional effects on diabetes which is a commonly associated comorbidity in NASH, based on streptozotocin-induced diabetic mouse model.

; An ideal drug candidate for NASH should reduce key clinical endpoints (i.e, steatosis and hepatic inflammation) and have antifibrotic effects , while also correcting underlying metabolic derangement. In this regard, EC 18 has shown additional beneficial effects on diabetes.

5. The liver may not be the primary organ to determine the bioavailability of EC-18, based on the general concept of dietary lipid metabolism and ADME of EC-18. Thus, EC-18 efficacy may be less affected by hepatic functional impairment associated with NASH.

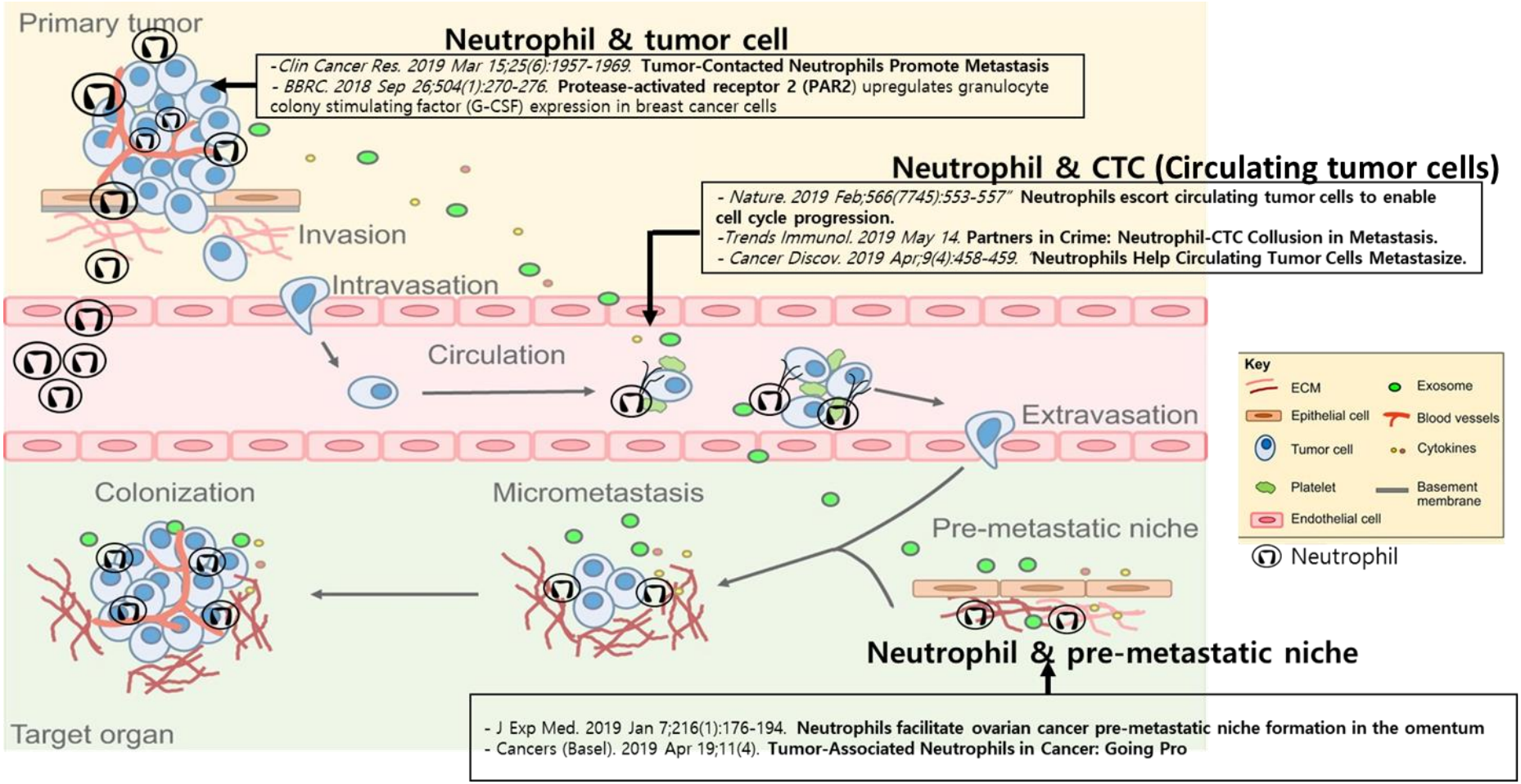
; Hepatic functional impairment often develops as NASH progresses to advanced fibrosis. If the liver is diseased, the lost liver function may interfere with a drug efficacy or safety depending on the PK/PD properties of the drug . EC 18 is transported via lymphatics after intestinal absorption (rather than entering the hepatic portal system and having first-pass effect in the liver) and the efficacy is minimally affected by impaired liver function, as opposed to other investigational agents which are metabolized or activated in liver tissue.

6. First-in-class MOA in NASH & Fibrosis

Healthy
and
Happy Life

Cancer Metastasis

Anti-metastatic Potentials of EC-18 via Neutrophil Trafficking Modulation



Summary

- EC-18 shows a synergistic anti-cancer effect when used in combination with AC regimen.
- EC-18 significantly reduces tumor infiltrating neutrophils via downregulation of chemokine expression
- EC-18 markedly reduces not only metastasis to brain and intestine but also primary lung cancer cell growth through reduction of tumor infiltrating neutrophils.
- EC-18 attenuates cancer cell invasiveness through inhibition of EMT activation by neutrophil.

EC-18 has the anti-tumor & anti-metastatic effects via reducing tumor infiltrating neutrophils & accelerating PAR2 degradation

2019 AACR-NCI-EORTC molecular targets conference selected abstract information (Oct 28)

ENZYCHEM LIFESCIENCES

The synergistic effect of PLAG on the anti-tumor efficacy of AC-regimen via alleviating neutrophil tumor infiltration on breast tumor xenograft model

Background: Tumor microenvironments (TME) promote tumor growth and induction of metastasis along with the strong presence of tumor-infiltrating neutrophil (TIN), which further contributes to abnormal tumor growth and metastasis. From this tumor-prone environment, we hypothesize that the effective destruction of TIN may enhance the therapeutic efficacy of chemotherapy for reducing the tumor. In this study, we investigated the synergistic effect of PLAG in the MDA-MB-231 breast cancer xenograft model concomitantly treated with the AC-regimen in modulating the effect of TIN.

Methods: MDA-MB-231 breast cancer xenograft model was used for evaluation of the tumor growth in the AC-regimen alone and PLAG co-treated animals. AC-regimen was delivered via intraperitoneal injection twice a week with a dose of 2/20 and 5/50 mpk (Doxorubicin/Cyclophosphamide) and PLAG was daily administered with 100 and 250 mpk. Tumor growth was measured in 3-day intervals. Neutrophil chemotaxis-related chemokines, CXCL1/2/8 and circulating neutrophils were also evaluated in a 2-week interval. Expression of apoptosis-related molecular markers (Bax/Bak) and TIN in the tumor lesion was analyzed by immunohistochemistry (IHC) staining.

Results: PLAG has synergistic effects on decreasing the tumor burden in the PLAG and AC-treated xenograft model. In AC-treated groups with 2/20 or 5/50 mpk, retardation of tumor growth was observed from the calculated tumor size and down-regulation of apoptosis-associated markers was proved by TUNEL assay and RT-PCR. Modulated chemokine expression from tumor burden and subsequent neutrophil recruitment were also detected in proportion to the tumor mass. The measured tumor sizes in the PLAG co-treated group were consistently smaller than those from the AC-regimen alone group till the mice were sacrificed. It was confirmed that the tumor burden of the PLAG co-treated group with 5/50 AC-regimen was significantly decreased in a concentration-dependent manner compared to the AC-regimen alone group ($p<0.05$). Surprisingly, in 250 mpk PLAG co-treated group, tumor seemed completely regressed on the sacrifice day. In accordance with the tumor size regression, significantly reduced chemokine expression and TIN in the PLAG co-treated group was demonstrated from the IHC and chemokine analysis. The tumor growth inhibition and reduced chemokine expression and TIN were also observed from the PLAG alone treated groups.

Conclusion: Taken together, PLAG has synergistic effects on relieving tumor burden concomitantly treated with AC-regimen. Combining the AC-regimen for the apoptotic effect of tumors and the PLAG treatment to regulate the number of circulating TIN will be a promising treatment modality for eliminating malignant tumors.

2019 AACR-NCI-EORTC molecular targets conference selected abstract information (Oct 29)

ENZYCHEM LIFESCIENCES

Anti-metastatic effect of PLAG via interference of neutrophil elastase/PAR2/EGFR activation pathway on A549 lung cancer orthotopic implantation model

Background: A recent study reported that tumor-infiltrating neutrophil (TIN) has potential on malignant tumor progression leading to metastasis. In our study, to control the effect of TIN, 1palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG), a synthetic lipid-based small molecular compound, was used. PLAG has shown its efficacy in modulating the neutrophil counts in chemotherapy-induced neutropenia by attenuating neutrophil extravasation via down-regulation of adhesion molecules, inflammatory cytokines, and chemokines. In this study, we investigated whether PLAG has an anti-metastatic effect on A549 lung cancer orthotopic implantation model via modulating TIN.

Methods: To investigate the anti-metastatic effect of PLAG on lung cancer metastasis, RFP-labeled A549 cells were implanted into the lungs of BALB/c nude mice via intratracheal injection and bred for 12 weeks. After 6 weeks from tumor implantation, PLAG with three concentrations (25/50/100 mpk) were daily administrated for another 6 weeks. Each image of the growth of the primary tumor and colonization of metastatic tumor to other sites were acquired using CT and IVIS. TIN was analyzed in the primary tumor using immunohistochemistry (IHC). To identify the mechanism of PLAG on preventing the neutrophil-associated metastasis of cancer cells, direct or non-direct co-culture methods were used.

Results: In the orthotopic implantation model, metastatic tumors to GI-track and brain were detected by IVIS. It was confirmed from the IVIS images that metastasis to GI-track was decreased by 73% in 100 mpk PLAG-treated group, while metastasis to the brain was decreased by about 92% in PLAG-treated group compared with positive control. Primary lung tumor growth was retarded by PLAG in a dose-dependent manner. The size of the primary tumor was much smaller than the positive control and alveolar tissue was similar to that of normal mice in 100 mpk PLAG-treated group. In contrast, massive neutrophil infiltrations in positive and delivery control groups were identified by IHC but significantly reduced upon PLAG administration. Moreover, p-EGFR was concomitantly detected with a similar pattern of neutrophil infiltration. During the direct contact in vitro assay, the A549 lung cancer spheroid cells were scattered by infiltrated neutrophils, but it was effectively hindered by PLAG treatment. Neutrophil-stimulated cancer in the non-direct contact method showed enhanced metastatic activity. However, such a phenomenon was significantly reduced with the PLAG-treated group. Moreover, neutrophil stimulated the expression of EMT markers, whereas it was effectively down-regulated by PLAG administration. Transactivation between tumor cells and neutrophil was mediated by neutrophil elastase (NE). NE binds to and activates protease-activated receptor 2 (PAR2) on cancer cells and subsequently phosphorylates EGFR for metastasis to occur by cleaving HB-EGF through the ARR2/clathrin complex. On the other hand, the suppression of metastasis by PLAG was mediated through the regulation of the NE-PAR2 pathway and acceleration of intracellular trafficking and degradation of PAR2

Conclusion: In this study, TIN was observed in the primary lung tumor and metastasis into GI track and brain, but PLAG dramatically reduced infiltration of neutrophil. The collective results suggest that TIN educates tumor cells for enhancing metastasis by EGFR transactivation. However, PLAG effectively interfered the transactivation cascade. Having not met the medical needs for treating metastasis, PLAG may be a promising candidate as an anti-metastatic drug by modulating the effect of TIN.

The Advantages of EC-18 in Cancer Metastasis

ENZYCHEM LIFESCIENCES

1. In terms of routes of drug administration, EC-18 has the advantage of being orally available drug, convenient for repeated and prolonged use. Oral dosing is imperative for the development of anti-metastatic drug because long-term drug administration is required for the prevention of metastasis.

2. No need for phase 1 clinical trial for drug development

3. The MOA of EC-18 targeting neutrophils for anti-metastasis is a common ground in the pathogenesis of metastasis of all epithelial origin cancers. EC-18 can be used in patients with solid cancers of epithelial origin, regardless of primary tumor sites.

4. EC-18 can be an attractive add-on therapy to any types of anti-cancer treatment modalities, such as chemotherapy, immunotherapy or molecularly targeted therapy for prevention and treatment of metastasis.

5. EC-18 does not interfere with anti-cancer effect of standard therapies for the primary tumors. Rather, EC-18 has a synergistic anticancer effect on primary tumor regression as well as metastatic tumors.

Healthy and Happy Life

Global Phase 2 Programs

ENZYCHEM LIFESCIENCES

Healthy
and
Happy Life

Chemoradiation-induced Oral Mucositis (CRIOM)

CONFIDENTIAL

DSMB Decision: Approves Stage 1 MTD: 2000mg

ENZYCHEM LIFESCIENCES

MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

Tucker Gosnell Center for Gastrointestinal Cancers
55 Fruit St, Yawkey Center 7E
Boston, Massachusetts 02114
Email: eroeland@mgh.harvard.edu
Tel: (617) 724-4000
Fax: (617) 726-0452

HARVARD MEDICAL SCHOOL

Eric Roeland, MD
Oncology & Symptom Science

May 31, 2019

To whom it may concern:

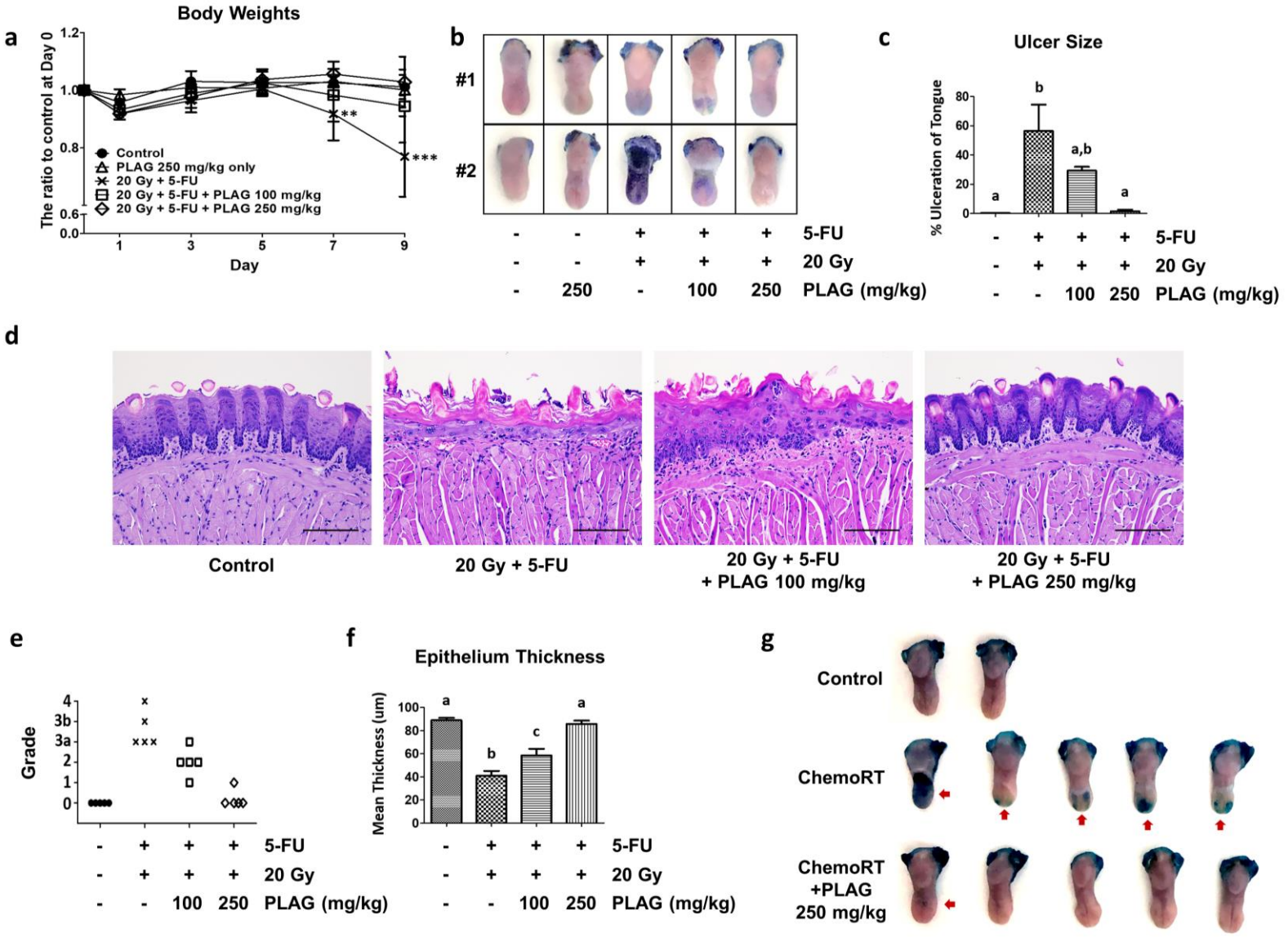
The iDSMB agree that the questions posed during iDSMB Data Review 2 have been satisfactorily answered and unanimously agree the Enzychem EC-18-202 trial may proceed as planned with Stage 2 dosing at 2000mg.

Sincerely,

Eric Roeland, MD
DSMB Chair

Animal Efficacy in CRIOM

ENZYCHEM LIFESCIENCES



Healthy
and
Happy Life

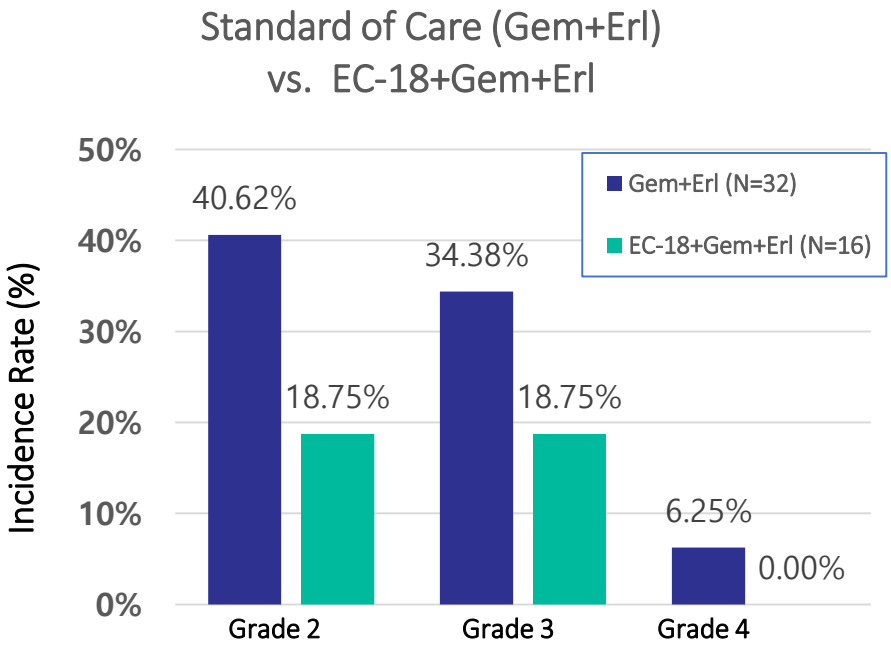
Chemotherapy-induced Neutropenia (CIN)

ENZYCHEM LIFESCIENCES

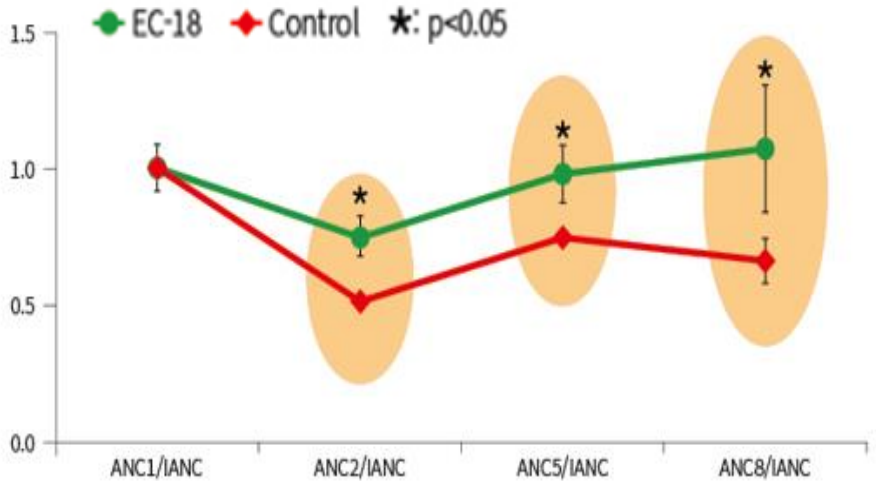
EC-18 Pilot Study in Pancreatic Cancer Patients

ENZYCHEM LIFESCIENCES

Study evaluated the effectiveness of EC-18 for the prevention of CIN in pancreatic cancer patients treated with gemcitabine-based chemotherapy



Total number of patients with Grade 2-4 neutropenia decreased 44.7% with addition of EC-18



Normal neutrophil range during 12 weeks of chemotherapy
No excessive increase of neutrophils

World J Oncol 6(4):410-415, 2015

EC-18 reduces the incidence of CIN in pancreatic cancer patients

AACR Annual Meeting 2019, Atlanta

ENZYCHEM LIFESCIENCES

Saving Lives One at A Time

ENZYCHEM LIFESCIENCES | KRIBB Korea Research Institute of Bioscience & Biotechnology

360

Therapeutic potential of EC-18 as a chemotherapy adjuvant for 5-fluorouracil-induced neutropenia

Yong-Jae Kim[1], Jinseon Jeong[1,2,3], Ki-Young Sohn[1], Do Young Lee[1], Sun Young Yoon[1], and Jae Wha Kim[2,3]

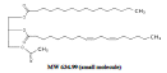
[1]Enzychem Lifesciences, Jecheon, Republic of Korea. [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea. [3] University of Science and Technology, Daejeon, Republic of Korea.

Abstract

Chemotherapy-induced neutropenia (CIN) is a complication that arises during cancer treatment and necessitates dose reduction. Preventing CIN and maintaining absolute neutrophil counts (ANC) is critical for successful chemotherapy because a rapid decline of neutrophils increases susceptibility to infection. Here, we investigated whether administration of EC-18 has therapeutic effects on the treatment of CIN in 5-fluorouracil (5-FU)-induced neutropenia mouse model. A single injection of 5-FU 100mg/kg reduced the ANC in the control, EC-18 125 and EC-18 250mg/kg-treated cohort from pre-injection values to <500 cells/μL by 5.2±0.45, 5.8±0.45 and 5.8±0.45 days, respectively. The administration of EC-18 in 5-FU-injected mice resulted in significant reduction in the duration of neutropenia and the time to recovery of ANC >1000 cells/μL. EC-18 125 or 250mg/kg significantly reduced the duration of neutropenia from 7.4±1.14 days to 2.6±0.55, 3.0±0.71 days, respectively. Moreover, the ANC of all individuals in the control cohort fell to severely neutropenic range (ANC <100 cells/ μL), while only 20% of individuals in both EC-18 125 and 250mg/kg-treated cohorts experienced severe neutropenia. EC-18 also reduced the duration of severe neutropenia from 5.2±1.48 days to 2 days. EC-18 125 or 250mg/kg administration significantly increased the mean nadir after 5-FU injection from 2.0±4.47 cells/μL to 236±4.47 or 158±11.32 cells/μL, respectively. The time of recovery to an ANC > 500 or 1000 cells/μL was significantly reduced in EC-18 125 and 250mg/kg-treated cohorts. Besides neutropenia, a single treatment of 5-FU induced the reduction of blood monocytes and eosinophils, similar to the pattern of the decrease of neutrophil counts. The administration of EC-18 125 or 250mg/kg in 5-FU-injected mice remarkably prevented the reduction of blood monocytes and eosinophils. In this study, thrombocytopenia is defined as a 50% or greater reduction in platelet count from baseline, and 2-fold or greater increase of platelet count from baseline for thrombocytosis. 5-FU treatment induced the moderate thrombocytopenia from 4 to 6 days and followed by a more pronounced and prolonged rebound thrombocytosis. EC-18 significantly reduced the extreme change in platelet counts, thus preventing 5-FU-induced thrombocytopenia and thrombocytosis. Moreover, EC-18 effectively prevented a constant reduction of red blood cell (RBC) count induced by 5-FU treatment. Based on the observations in this study, we concluded that EC-18 has therapeutic potential as a chemotherapy adjuvant for the treatment of 5-FU-induced CIN as well as chemotherapy-associated other hematologic disorders.

Introduction

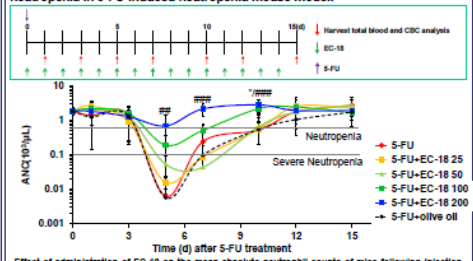
- Chemotherapy-induced neutropenia (CIN) is a complication that arises during cancer treatment and necessitates dose reduction. **Caggiano V, Weiss RV, Rickert TS, Linde-Zwirble WT. Cancer 2005;103:1916-24.**
- Preventing CIN and maintaining absolute neutrophil counts is critical for successful chemotherapy because a rapid decline of neutrophils increases susceptibility to infection. **Santolaya ME, Alvarez AM, Becker A, Cofre J, Enriquez N, O'Ryan M, *et al.* J Clin Oncol 2001;19:3415-21**
- In previous study, EC-18 attenuated gemcitabine-induced neutropenia via regulation of neutrophil extravasation. **Jeong *et al.* Cell Biosci. 2019; 9: 4. (Published online 2019 Jan 3. doi: 10.1186/s13578-018-0266-7).**



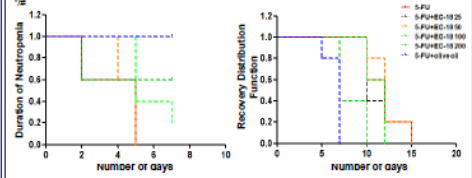
EC-18 (PLAG: 1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol)

Results

1. Therapeutic effect of administration of EC-18 on the treatment of neutropenia in 5-FU-induced neutropenia mouse model.



Effect of administration of EC-18 on the mean absolute neutrophil counts of mice following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; */#



Effect of administration of EC-18 on the duration of neutropenia and on time to recovery to ANC <500 cells/μL.

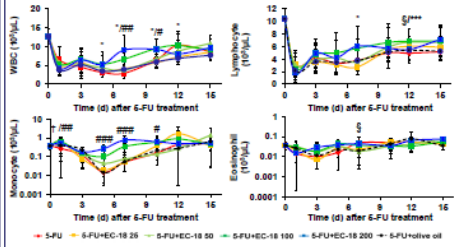
Treatment	Mean First Day Neutropenia (± SE, range)	Mean Duration of Neutropenia in Days (± SE, range)	Number of Individuals of Severe Neutropenia	Mean Duration of Severe Neutropenia in Days (± SE, range)
Control	4.8±0.4 (3-5)	6.8±0.75 (5-8)	5/5	3.8±0.7 (2-5)
EC-18 25mg/kg	3.8±0.49 (3-5) (*P* = NS)	8.4±1.17 (6-12) (*P* = NS)	5/5	4.8±0.2 (4-5)
EC-18 50mg/kg	5.0±0.0 (5-5) (*P* = NS)	8.2±0.80 (6-7) (*P* = NS)	4/5	5.5±0.5 (5-7)
EC-18 100mg/kg	5.4±0.4 (5-7) (*P* = NS)	2.8±0.80 (2-5) (*P* = 0.0031)	2/5	2.0±0.0 (2-2)
EC-18 200mg/kg	4.8±0.4 (3-5) (*P* = NS)	2.0±0.0 (2-2) (*P* = 0.001778)	0/5	N/A
Olive oil	5.0±0.0 (5-5) (*P* = NS)	6.8±0.49 (6-7) (*P* = NS)	5/5	3.8±0.7 (2-5)

Table 1. Mean First Day of Neutropenia (ANC <500 cells/μL), Mean Duration of Neutropenia, Number of Individuals of Severe Neutropenia (ANC <100 cells/μL), and Mean Duration of Severe Neutropenia in Control, and EC-18 25, 50, 100, 200 and olive oil-treated mice injected with 5-FU 100mg/kg

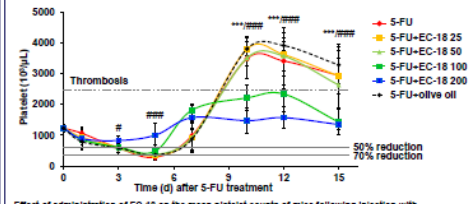
Treatment	Nadir of ANC (cells/μL)	Mean Number of Days to Recovery – ANC ≥500/μL (± SE, range)	Mean Number of Days to Recovery – ANC ≥1000/μL (± SE, range)
Control	8±6.0	11.8±0.9 (10-16)	12.8±0.8 (12-16)
EC-18 25mg/kg	14±2.4 (*P* = NS)	12.2±0.8 (10-16) (*P* = NS)	12.2±0.8 (10-16) (*P* = NS)
EC-18 50mg/kg	42±22.9 (*P* = NS)	11.2±0.5 (10-12) (*P* = NS)	10.0±0.0 (10-10) (*P* = 0.0081)
EC-18 100mg/kg	168±76.0 (*P* = NS)	8.2±0.7 (7-10) (*P* = 0.0156)	10.0±0.0 (10-10) (*P* = 0.0081)
EC-18 200mg/kg	368±52.2 (*P* = 0.0002)	6.8±0.4 (6-7) (*P* = 0.0008)	7.2±0.8 (6-10) (*P* = 0.0008)
Olive oil	8±3.7 (*P* = NS)	10.8±0.5 (10-12) (*P* = NS)	12.8±1.0 (10-16) (*P* = NS)

Table 2. Mean Nadir and Recovery from Neutropenia in Control, EC-18 25, 50, 100, 200 and olive oil-Treated mice injected with 5-FU 100mg/kg.

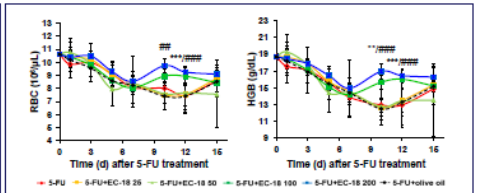
2. Therapeutic effect of administration of EC-18 on other hematologic indicators in 5-FU-treated mouse model



Effect of administration of EC-18 on the mean WBC, lymphocyte, monocyte and eosinophil counts of mice following injection with 100mg/kg of 5-fluorouracil. †§-FU vs. 5-FU+EC-18 25 mg/kg, §§-FU vs. 5-FU+EC-18 50 mg/kg,* 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; †/§/*/#P<0.05, ††/§§/**/##P<0.01 and †††/§§§/***/###P<0.001.

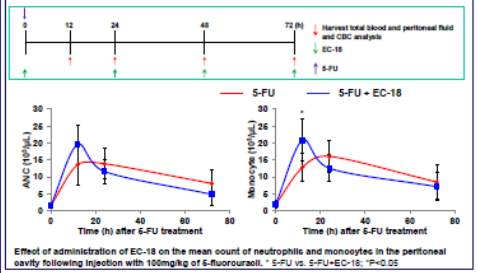


Effect of administration of EC-18 on the mean platelet counts of mice following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; */#P<0.05, **/##P<0.01 and ***/###P<0.001.



Effect of administration of EC-18 on the mean RBC counts and hemoglobin levels of mice following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; */#P<0.05, **/##P<0.01 and ***/###P<0.001.

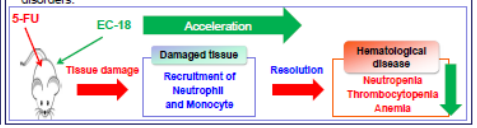
3. Effect of EC-18 administration on leukocyte recruitment in 5-FU-treated mouse model



Effect of administration of EC-18 on the mean count of neutrophils and monocytes in the peritoneal cavity following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18; *P<0.05

Conclusion

- Under 5-FU-induced neutropenic condition, EC-18 significantly increased the ANC and reduced the duration of neutropenia and time of recovery.
- EC-18 also effectively prevented other hematologic disorders induced by 5-FU treatment, such as the reduction of blood monocytes and eosinophils, thrombocytopenia, thrombocytosis and anemia.
- Based on the observations in this study, we concluded that therapeutic administration of EC-18 could be developed as a chemotherapeutic adjuvant for the treatment of CIN as well as chemotherapy-associated other hematologic disorders.



2019 AACR-NCI-EORTC, Boston (Oct. 27)

A phase 1b/2, open-label, multicenter dose-escalation study of EC-18 in patients with metastatic breast cancer for the prevention of chemotherapy-induced neutropenia

Background/Aim: Chemotherapy-induced neutropenia (CIN) is the major dose-limiting toxicity of systemic cancer chemotherapy leading to dose reductions, treatment delays or treatment early termination, which may compromise treatment outcome. EC-18 is a synthetic, orally available and lipid-based small molecular compound (1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol). EC-18 prevents CIN by attenuating neutrophil extravasation via down-regulation of adhesion molecules, proinflammatory cytokines & chemokines (Cell & Bioscience 2019;9:4). The aim of this study is to evaluate the safety, tolerability, pharmacokinetics (PK) and preliminary activity of EC-18 for CIN (NCT03104595).

Healthy
and
Happy Life

US Government Funding Programs (ARS)

ARS: Funding Opportunities

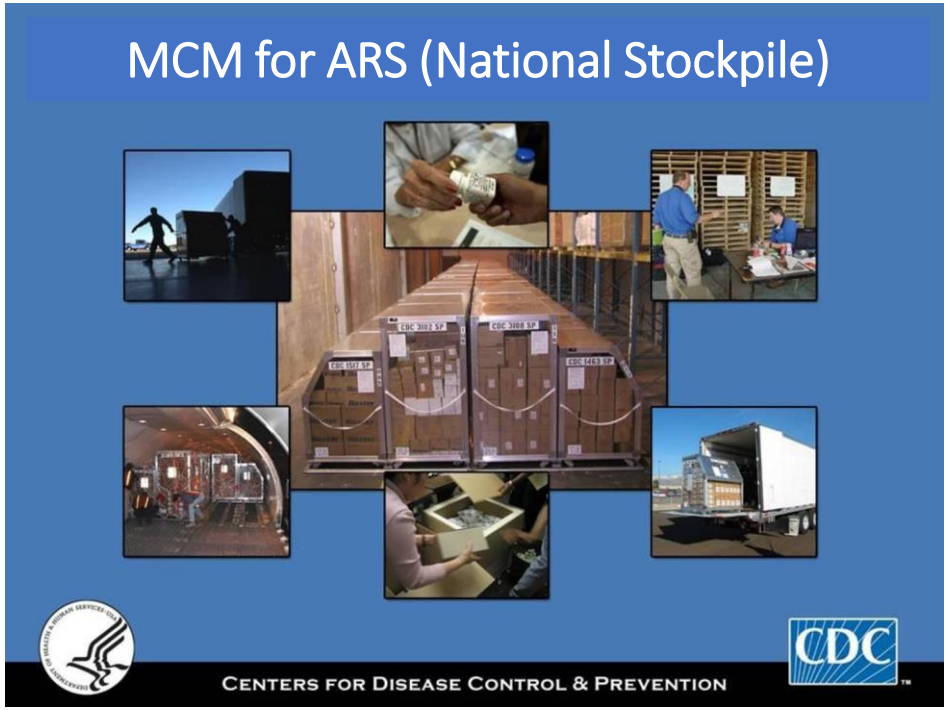
ENZYCHEM LIFESCIENCES

ARS Drug R&D Funding

BARDA — Biomedical Advanced Research and Development Authority

NIH — National Institute of Allergy and Infectious Diseases

Department of Defense — United States of America



*EC-18 was awarded RNCP (Radiation and Nuclear Countermeasures Program) & CCRP (Chemical Countermeasures Research Program) program by NIAID (National Institute of Allergy and Infectious Diseases)

In Active Discussion for Funding from BARDA, NIAID, DoD, and NASA

AACR Annual Meeting 2019, Atlanta

ENZYCHEM LIFESCIENCES

Saving Lives One at A Time

ENZYCHEM LIFESCIENCES | KRIBB Korea Research Institute of Bioscience & Biotechnology

3730

1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol mitigates the hematopoietic syndrome of lethal acute radiation syndrome in mice

Yong-Jae Kim[1], Jinseon Jeong[1,2,3], Su-Hyun Shin[2,3], Ki-Young Sohn[1], Do Young Lee[1], Sun Young Yoon[1], and Jae Wha Kim[2,3]

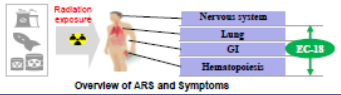
[1]Enzychem Lifesciences, Jecheon, Republic of Korea. [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea. [3] University of Science and Technology, Daejeon, Republic of Korea.

Abstract

The acute radiation syndrome (ARS) is a broad term used to describe a range of signs and symptoms that reflect severe damage to specific organ systems and that can lead to death within hours to several months after exposure. In this study, we investigated the efficacy of EC-18 for the development of a medical countermeasure for ARS by analyzing ionizing radiation (IR)-induced mortality and morbidity. First, we established a murine model of the ARS by exposing eleven week old male and female BALB/c mice to 6.0-6.5Gy doses of total body irradiation (TBI; γ-ray, ^{60}Co, 1553R/min), and assessed for 30 day survival, mean survival time and lethality dose (LD). The $LD_{70/30}$ with confidence interval (CI) was 6.11Gy (5.98-6.22Gy). To determine the efficacy of EC-18 in IR-induced mortality, we exposed BALB/c mice to a 6.11Gy dose ($LD_{70/30}$) of TBI and orally administered 10-250 mg/kg/day of EC-18, starting one day after irradiation. As a result, 6.11Gy of γ-radiation caused the death of 80% of the animals of positive control group within 23days, with an average life span (ALS) of 17.9days. The percentages of survival of the irradiated mice with EC-18 10, 50, and 250mg/kg were 20%, 40%, and 80% with ALS of 19.3, 22.3, and 28.2days, respectively. Moreover, the LD70/30 dose of γ-ray irradiation caused a substantial decrease in the body weight of the mice. The administration of EC-18 effectively prevented severe weight loss induced by irradiation. Next, we investigated the efficacy of EC-18 for hematopoietic ARS (H-ARS) by analyzing the kinetics of white blood cells (WBC), red blood cells (RBC), and platelets. A single whole body exposure of γ-radiation (6.11Gy) rapidly exhausted all kinds of WBC counts, and the administration of EC-18 significantly attenuated γ-radiation-induced depletion of WBCs in the irradiated mice. Especially, the administration of EC-18 substantially reduced γ-radiation-induced reduction of the absolute neutrophil counts (ANC). The mean first day of neutropenia (ANC<500cells/μL) of control and EC-18-treated cohorts was 1.8±1.09 and 2.2±1.09 days, respectively. Although EC-18 did not protect the irradiated mice from experiencing severe neutropenia, it effectively reduced the duration of severe neutropenia from 13.0 days to 7.2±1.79days. In addition, EC-18 significantly increased the mean nadir of ANC after γ-ray irradiation from 4.0±5.48 cells/μL to 20.0±10.00 cells/μL. In addition, the administration of EC-18 in the irradiated mice remarkably attenuated the rapid reduction of RBCs and hemoglobin. When exposed to a supra-lethal dose (8Gy) of γ-radiation, the two of five mice in the control cohort experienced severe skin discoloration and edema formation on the front right feet and hemorrhagic telangiectasia on the tales on day10. EC-18 remarkably improved γ-radiation-induced skin damage in the irradiated mice. Based on the observations in this study, we concluded that EC-18 has potential as a medical countermeasure for ARS.

Introduction

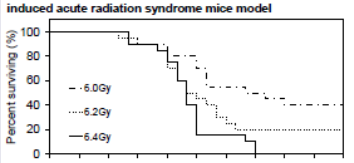
- The acute radiation syndrome (ARS) is a broad term used to describe a range of signs and symptoms that reflect severe damage to specific organ systems and that can lead to death within hours or up to several months after exposure. The ARS occurs after whole-body or significant partial-body irradiation of greater than 1 Gy, over a short time period (high dose rate). **López, Mario, and Margarita Martin. Reports of Practical Oncology & Radiotherapy 16.4 (2011): 138-146.**
- Since the risk of exposure to radiation continues to increase, there has also been an increasing interest in the search of ways of protection against the effects of acute irradiation by ionizing radiation in accidental condition. **Aminin, Dmitry L., et al. Natural product communications 6.5 (2011): 587-592.**



Overview of ARS and Symptome

Results

1. Determination of Lethal Dose (LD)XX/30 in γ-radiation-induced acute radiation syndrome mice model

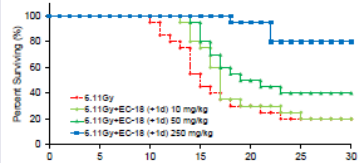


Survival rates of BALB/c mice. BALB/c mice (11 week old, male and female) exposed to ^{60}Co source of γ-radiation. Kaplan-Meier survival curves showing the proportion of mice surviving at each time points for each radiation dose of γ-ray.

LD XX/30	LD estimate (Gy)	Lower 95% CI (Gy)	Upper 95% CI (Gy)
LD30/30	5.31	4.98	5.56
LD50/30	5.79	5.59	5.96
LD70/30	6.11	5.98	6.22
LD95/30	6.39	6.30	6.48

Table 1. Estimated lethal dose in BALB/c mice after ^{60}Co γ-radiation.

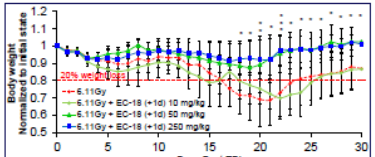
2. Dose effect relationship of EC-18 on the survival rate and body weight loss under γ-ray-induced acute radiation syndrome (ARS)



Dose effect of EC-18 administration on survival rates of mice irradiated with a dose of 6.11Gy of γ-radiation.; *P<0.0011, 6.11Gy + EC-18 50mg/kg versus 6.11Gy; *P<0.0001, 6.11Gy + EC-18 250mg/kg versus 6.11Gy (Log rank test)

Treatment	Mice Survived /total	Survival rate	Mean survival time (days)	Median Survival (days)	Log-rank test P*
6.11Gy	4/20	20%	17.9	15	
6.11Gy + EC-18 10mg/kg	4/20	20%	19.3	17	0.4425
6.11Gy + EC-18 50mg/kg	8/20	40%	22.3	20	0.0464
6.11Gy + EC-18 250mg/kg	16/20	80%	28.2	30	<0.0001

Table 2. Dose effect relationship of EC-18 on survivability and average life duration of irradiated mice

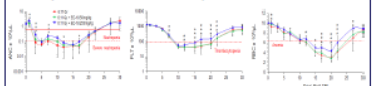


Effects of administration of EC-18 on body weights of irradiated mice. Normalized body weights of irradiated mice with a 6.11Gy dose of γ-radiation.

Treatment	10% Body Weight Loss		20% Body Weight Loss	
	n	%	N	%
6.11Gy	16	80%	8	40%
6.11Gy + EC-18 10mg/kg	17	85%	14	70%
6.11Gy + EC-18 50mg/kg	11	55%	7	35%
6.11Gy+ EC-18 250mg/kg	3	15%	3	15%

Table 3. PLAG significantly mitigates body-weight loss in mice exposed to the LD70/30 dose of γ-radiation.

3. PLAG mitigates the depletion of ANC, PLT, RBC, HGB in mice exposed to LD70/30 dose of γ-radiation.



EC-18 showed efficacy in improving neutropenia, thrombocytopenia and anemia in 24 h-delayed treatment model.

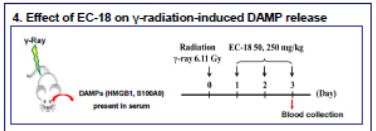
Treatment	Severe Neutropenia (ANC < 10^3 cells/μL)		Thrombocytopenia (PLT<10^4 cells/μL)		Anemia (HGB < 12 g/dL)	
	Mean First Day (±SE, range)	Mean Duration in Days (±SE, range)	Mean First Day (±SE, range)	Mean Duration in Days (±SE, range)	Mean First Day (±SE, range)	Mean Duration in Days (±SE, range)
6.11Gy	5.6±0.9 (3-17)	14.5±1.6 (4-23)	11.7±0.2 (7-12)	11.6±1.3 (5-18)	11.3±0.6 (3-17)	16.4±1.1 (10-18)
6.11Gy + EC-18 50mg/kg	9.0±1.1 (3-17) (P < 0.0271)	11.4±1.4 (4-19) (P = NS)	12.0±0.0 (12-12) (P = NS)	12.5±1.3 (5-18) (P = NS)	12.5±0.3 (12-17) (P = NS)	15.1±1.5 (5-18) (P = NS)
6.11Gy + EC-18 250mg/kg	12.1±0.8 (3-17) (P < 0.0001)	8.8±0.7 (5-14) (P = 0.0025)	12.0±0.0 (12-12) (P = NS)	6.0±0.5 (5-10) (P < 0.0001)	14.5±0.6 (12-17) (P = 0.0005)	9.1±1.0 (5-18) (P = 0.0004)

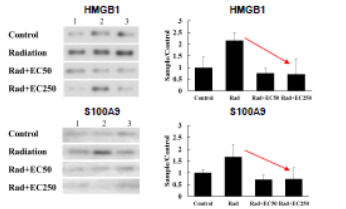
Table 4. Mean first day and mean duration of severe neutropenia (ANC <100 cells/μL), thrombocytopenia (PLT <100 x 10^3 cells/μL) and nemia (HGB <12 g/dL) in control and EC-18-treated mice exposed to lethal radiation dose

Treatment	Nadir of ANC (cells/μL)	Mean Number of Days to recovery ANC ≥ 500cells/μL (±SE, range)	Nadir of platelet (10^3 cells/μL)	Mean Number of Days to recovery platelet ≥ 10^4cells/μL (±SE, range)	Nadir of RBC (10^6 cells/μL)	Mean Number of Days to recovery RBC ≥ 6.3 x 10^6 cells/μL (±SE, range)
6.11Gy	29.0±4.3	25.2±1.9 (22-30)	35.4±3.7	23.2±1.3 (17-30)	3.8±0.4	28.0±1.3 (22-30)
6.11Gy + EC-18 50mg/kg	42.0±6.3 (P = NS)	25.4±1.1 (22-30) (P = NS)	38.0±3.5 (P = NS)	24.5±1.3 (17-30) (P = NS)	3.7±0.2 (P = NS)	29.1±0.9 (22-30) (P = NS)
6.11Gy + EC-18 250mg/kg	72.5±5.2 (P < 0.0001)	26.2±0.9 (22-30) (P = NS)	58.5±4.2 (P = 0.0002)	18.0±0.5 (17-22) (P = 0.0002)	4.8±0.2 (P = 0.0225)	25.3±1.0 (22-30) (P = NS)

Table 5. Mean nadir and mean number of days to reoovery of ANC, platelets and RBCs in control and EC-18-treated mice exposed to lethal radiation dose

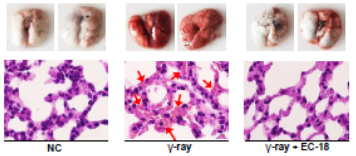
4. Effect of EC-18 on γ-radiation-induced DAMP release





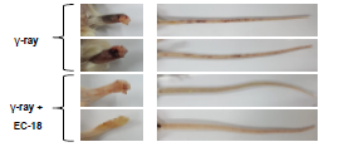
Effect of EC-18 administration on the release of HMGB1 and S100A9 in blood of mice irradiated with a dose of 6.11Gy of γ-radiation.

5. Effect of EC-18 on γ-radiation-induced lung injury.



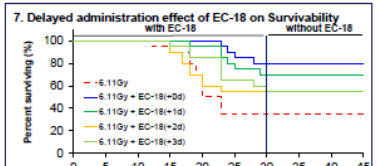
Effect of EC-18 administration on vasoular leakage in lung of mice irradiated with a dose of 6.11Gy of γ-radiation.

6. Effect of EC-18 on γ-radiation-induced hemorrhagic telangiectasia and edema.



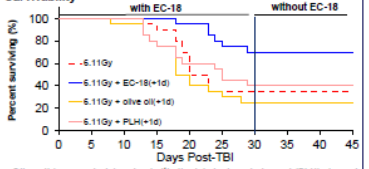
Effect of EC-18 administration on skin discoloration and edema formation of mice irradiated with a dose of 8Gy of γ-radiation.

7. Delayed administration effect of EC-18 on Survivability



Radiation only vs. EC-18 (250mpk) co-treatment, *** $P < 0.001$; Radiation only vs EC-18 24h post-IR, *** $P < 0.001$; Radiation only vs EC-18 48h post-IR, ** $P < 0.01$; Radiation only vs EC-18 72h post-IR, ** $P < 0.01$

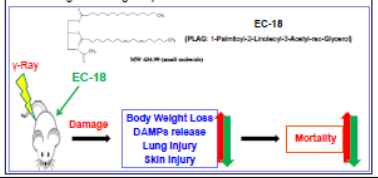
8. Comparison of EC-18 with olive oil and PLH on Survivability



Olive oil (same calorie) and palmitic linoleic hydroxyl glycerol (PLH) showed little effect on survival in 24h delayed treatment. EC-18 has a distinctive mechanism of action for improving survivability in γ-radiation-induced ARS.

Conclusion

- Under γ-radiation-induced ARS condition, the administration of EC-18 significantly attenuated the radiation-associated mortality and loss of body weight in a dose-dependent manner.
- γ-radiation induced the rapid exhaustion of all kinds of blood cells, which is defined by γ-radiation-induced hematopoietic injury. The administration of EC-18 significantly attenuated γ-radiation-induced reduction of ANC, PLT and RBC counts.
- Based on the observations in this study, we concluded that EC-18 has therapeutic potential for improving survivability and reducing hematological damage in γ-radiation-induced ARS.



Healthy and Happy Life

Appendix

ENZYCHEM LIFESCIENCES

Publications

ENZYCHEM LIFESCIENCES

	Target indications/ Pharmacological effects	Journal	Year
1	Acute radiation syndrome	Radiation Research	2019
2	MOA (Phagocytosis/Acute lung injury)	Frontiers in Immunology	2019
3	Diabetes	Mol Cell Biol	2019
4	MOA (Extravasation of neutrophil/Neutropenia)	Cell & Bioscience	2019
5	Thrombocytopenia	Thrombosis Research	2018
6	Hepatitis	J Cell Biochem	2018
7	Rheumatoid arthritis	Oncotarget	2017
8	Cognitive dysfunction	Behavioural brain research	2017
9	Oral mucositis	Frontiers in Oncology	2016
10	Neutropenia	Cancer Letters	2016
11	Asthma	PLoS One	2016
12	Chronic obstructive pulmonary disease	Int. Immunopharm.	2016
13	Neutropenia	World J Oncol	2015
14	Immune regulation	Immune Network	2015
15	Atopic dermatitis	Immune Network	2015
16	Asthma	Int. Immunopharm	2014
17	Sepsis	J. Trauma	2010
18	Cancer metastasis	J Korean Med Sci	2009
19	Hematopoiesis	Biol Pharm Bull	2004
20	Hematopoiesis	Chem Pharm Bull	2004

Paper 1

1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol (PLAG) Rapidly Resolves LPS-Induced Acute Lung Injury Through the Effective Control of Neutrophil Recruitment

Acute lung injury (ALI) is an acute respiratory failure that is associated with excessive neutrophil recruitment and high mortality. To assess the efficacy of 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) as a therapeutic agent for ALI, this compound was administered orally to mice challenged with an intranasal dose of lipopolysaccharide (LPS). Using this model, we found that PLAG promotes resolution of ALI through effective control of LPS-induced neutrophil infiltration, endothelial permeability, and inflammatory chemokine production. In addition, the Toll like Receptor 4 (TLR4) endocytosis/exocytosis cycle was significantly accelerated in Raw 264.7 cells co-treated with PLAG/LPS, as compared to cells treated only with LPS. During this cycle, a PLAG-induced exotoxin clearance pathway was observed to occur through the prompt assemblyofnicotinamideadeninedinucleotidephosphate (NADPH)unitsandproduction of reactive oxygen species (ROS), which ultimately lead to earlier LPS clearance. We further detected reduced expression, as well as faster return to homeostatic levels, of macrophage inflammatory protein (MIP)-2, in PLAG/LPS- vs. LPS-treated cells. MIP-2 is a main inducer of neutrophil migration that is mainly controlled by interferon regulatory factor 3 (IRF3) activation and is involved in the TLR4 endosomal-signaling pathway. PLAG induced TLR4-mediated TRIF-related adaptor molecules/Toll-interleukin receptor (TIR) domain-containingadaptorproteinincludinginterferon(IFN)-β/IRF3endosomalsignaling, leading to rapid association of TRAM/TRIF and TLR4 and earlier IRF3 phosphorylation in PLAG/LPS-treated vs. LPS-treated cells. PLAG specificity was further verified with PLAG analogs and metabolites known to control excessive neutrophil infiltration, suggesting that this acetylated diacylglycerol has a unique biological role in neutrophil motility. Thus, our data indicate that PLAG may represent a potential therapeutic agent for resolution of LPS-induced lung inflammation through effective MIP-2 modulation.

Frontiers in Immunology | www.frontiersin.org
September 2019 | Volume 10 | Article 2177

Paper 2

PLAG Attenuates STZ-Induced Pancreatic Beta Cell Damage by Promoting GLUT2 Endocytosis

Streptozotocin (STZ) is widely used to induce diabetic rodent models. It is specifically toxic to pancreatic beta cells and causes severe destruction and dysfunction. We investigated the effect of 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) on an STZ-induced diabetic mouse model. PLAG attenuated the glucose increase and maintained serum insulin levels similar to control mice. In the pancreatic beta cell line INS-1, STZ-induced cell apoptosis and intracellular reactive oxygen species (ROS) generation were significantly reduced to nearly normal levels after PLAG treatment. Glucose transporter 2 (GLUT2) localization analyses and glucose uptake assays showed that PLAG accelerated GLUT2 internalization, which ameliorated excessive entry of glucose, as well as STZ. STZ-induced cytotoxic effects were significantly reduced in PLAG treated groups. The biological activity of PLAG was further confirmed in GLUT2-silenced cells, and the specificity of PLAG was verified using its derivative, 1-palmitoyl-2-linoleoyl-3-hydroxyl rac-glycerol (PLH). Our results suggest that PLAG may be a useful agent for protecting beta cells in the setting of excessive glucose influx.

Molecular and Cellular Biology
November 2019, Volume 39, No 21

Paper 3

Mitigating Effects of 1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol (PLAG) on Hematopoietic Acute Radiation Syndrome After Total-body Ionizing Irradiation in Mice

Acute radiation syndrome (ARS) is an acute illness caused by irradiation of the entire body or most of the body by a high dose of penetrating ionizing radiation (IR) in a very short period of time. The severity of hematopoietic sub-syndrome of ARS in irradiated individuals has known to be associated with survival. The purpose of this study is to investigate the therapeutic effects of a lipid molecule, 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG), on the kinetics of hematopoietic cells, including absolute neutrophil count (ANC), red blood cells (RBCs), and platelet counts, in mice after exposure to total-body irradiation (TBI) with gamma radiation. Male and female BALB/c mice (11 weeks old) were exposed to an LD70/30 dose of TBI. PLAG significantly and dose-dependently attenuated gamma radiation-induced mortality ($p=0.0041$ for PLAG 50 mg/kg; $p<0.0001$ for PLAG 250mg/kg) and body-weight loss ($p<0.0001$ for PLAG 50 and 250 mg/kg) in mice. A single TBI of gamma radiation sharply reduced ANC within 3 days after irradiation and maintained the neutropenic state (ANC < 500 cells/μL) by about 26.8 ± 0.8 days. In particular, administration of PLAG attenuated gamma radiation-induced severe neutropenia (ANC < 100 cells/μL) by effectively delaying the mean day of its onset and decreasing its duration. PLAG also significantly mitigated gamma radiation-induced thrombocytopenia ($p<0.0001$ for PLAG 250mg/kg) and anemia ($p=0.0023$ for PLAG 250mg/kg) by increasing mean platelet and RBC counts, as well as hemoglobin levels, in peripheral blood. Moreover, delayed administration of PLAG, even at 48 and 72 h after IR exposure, significantly attenuated gamma radiation-induced mortality in a time-dependent manner. When compared to olive oil and palmitic linoleic hydroxyl (PLH), only PLAG effectively attenuated gamma radiation-induced mortality, indicating that it has a distinctive mechanism of action. Based on these preclinical observations, we concluded that PLAG has high potential as a radiation countermeasure for the improvement of survivability and the treatment of hematopoietic injury in gamma radiation-induced ARS.

Radiation Research Accepted

Paper 4

1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol (PLAG) Attenuates Gemcitabine-Induced Neutrophil Extravasation

Cancer patients treated with chemotherapy often experience a rapid decline of blood neutrophils, a dose-limiting side effect called chemotherapy-induced neutropenia. This complication brings about dose reductions or cessation of chemotherapy during treatment of cancer patients because a rapid decline of neutrophil counts increases susceptibility to infection. Here, we found that 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) attenuates gemcitabine- induced neutrophil extravasation via the inhibition of neutrophil-attracting chemokine production in macrophages using in vivo and in vitro approaches. A single intraperitoneal administration of gemcitabine induced the migration of circulating neutrophils into the peritoneal cavity in normal mice, and PLAG effectively decreased neutrophil migration by inhibiting the expression of adhesion molecules, L-selectin and LFA-1. Inhibition of CXCR2 by its antagonist, reparixin, abrogated gemcitabine-induced neutrophil migration, indicating that chemokines produced by gemcitabine mainly support neutrophil activation. In vitro experiments demonstrated that PLAG inhibited NADPH oxidase 2 (NOX2)-mediated reactive oxygen species production induced by gemcitabine, which is the upstream of MIP-2 and/ or CXCL8. Importantly, PLAG down-regulated gemcitabine-induced membrane translocation of the cytosolic NOX subunit, Rac1, and phosphorylation of p47phox. The activation of upstream signaling molecules of p47phox phosphorylation, phospholipase C β3 and protein kinase C, were effectively regulated by PLAG. We also demonstrated that 1-palmitoyl-2-linoleic-3-hydroxyl-rac-glycerol (PLH), the natural form of diacylglycerol, has no effects on gemcitabine- induced CXCL8 production and dHL-60 migration, suggesting that an acetyl group at the third position of the glycerol backbone may have a key role in the regulation of neutrophil activation. Altogether, this study suggests the potential of PLAG as a therapeutic strategy to modulate chemotherapy-induced neutrophil activation for cancer patients undergoing chemotherapeutic treatment.

Cell & Bioscience
Jan. 2019, Volume 9, No 4

Papers in preparation

	Target indications/ Pharmacological effects	Target Journal
1	MOA (Efferocytosis)	FEBS
2	CRIOM	Oral Disease
3	Psoriasis	Int. J. Mol. Sci.
4	Anti-metastasis	
5	Synergistic anticancer effect	
6	NASH	

Intellectual Property

	Registered/Pending	Total No.	Registered/Application in 2019
EC-18	Registered	87	10
	Pending	64	23
API	Registered	11	0
	Pending	3	1
Contrast Agents	Registered	1	1
	Pending	9	2

Registered IP in 2019

ENZYCHEM LIFESCIENCES

No.	Country	IP
1	IN	Preparation method of 1-Palmitoyl-3-acetylglycerol, and preparation method of 1-Palmitoyl-2-Linoleoyl-3-Acetyl glycerol using same
2	CN	Chronic Obstructive Pulmonary Diseases (COPD)
3	EP	
4	CA	Leukopenia and thrombocytopenia
5	EP	
6	RU	
7	EP	Rheumatoid Arthritis (RA)
8	CN	Blood Cancer or Metastasis
9	EP	
10	EP	Atopic Dermatitis

New Application in 2019

ENZYCHEM LIFESCIENCES

No.	Country	IP
1	CN	Blood Cancer or Metastasis
2	AU	Psoriasis
3	CN	
4	US	
5	EP	
6	CA	
7	JP	
8	IN	
9	AU	Hepatitis
10	CN	
11	US	
12	EP	
13	CA	
14	JP	
15	IN	
16	PCT	Diacylglycerol lactone compound, method for preparing the same and immunity enhancing agent including the same as active ingredient
17	PCT	immunomodulating agent including the same as active ingredient
18	PCT	Glycerol derivative, method for preparing the same and immunomodulating agent including the same as active ingredient
19	US	Composition and Methods for Modulating an Inflammatory Response
20	PCT	Methods and Compositions for Treatment of Cancer
21	KR	NASH
22	KR	Diabetes mellitus
23	KR	Gout


Thank You