BIOTECH SUPPORT GROUP
Sample Prep that Matters

CLEANASCITE™ & BINDPRO™

LIPID/PROTEIN REMOVAL & CELL RESPONSE APPLICATIONS

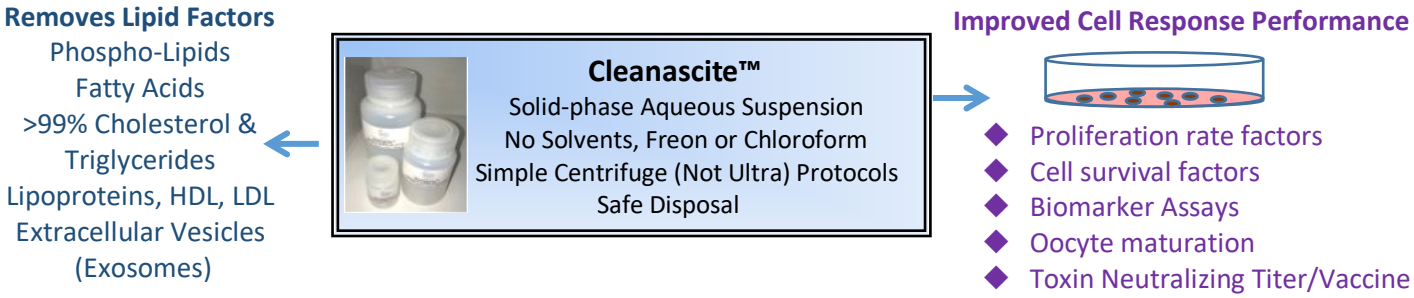
FEBRUARY 21, 2023

Introduction

The "omics" revolution demanded new and different sample prep separations that were not efficiently performed by conventional technologies. While effective for many applications, these tools were not efficient for "omics" sample preparation, as throughput, economy and simplicity are especially required. Furthermore, these same separation tools often denatured proteins which limited there use in applications which demanded the measurement of function, structure or bio-activity.

For these reasons, BSG has been dedicated to create new methods and applications to drive efficient workflows and better data quality for all proteomic and biomarker analyses. Of special importance is the value created when certain families of biomolecules can be evaluated with respect to cell response and viability. For example, extracellular vesicles (EVs) substantially influence cultured cell behavior. While all of our products can serve cell response applications, we report here an extensive list of applications in this area for **Cleanascite™ & BindPro™**.

Cleanascite™ is derived through a proprietary formulation of metallic oxide derivatives. However, unlike other metallic oxides, **Cleanascite™** does not have significant protein binding, making its selectivity profile for lipids un-rivaled in the bio-research products industry. As a result, it is ideal to clear lipid-associated matrix effects - including extracellular vesicles, which may influence cell response assays.



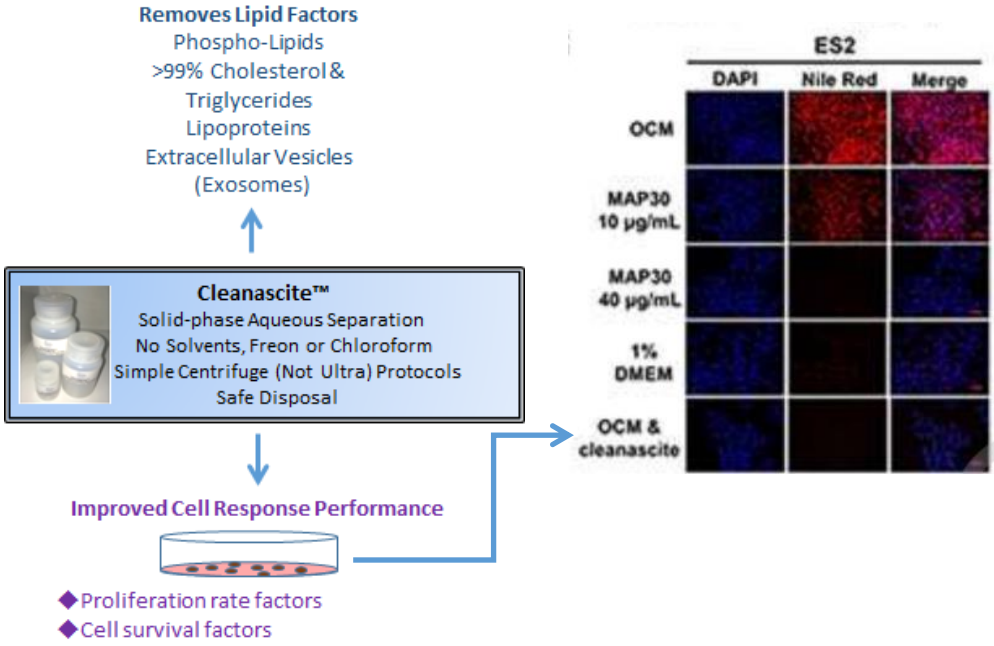
Cleanascite™ has been validated in accordance with CLIA '88 guidelines. The applications and references for the many diverse investigations using **Cleanascite™** upstream of cell response measurements are next described.

BindPro™ is a polymeric protein removal suspension reagent, designed to very efficiently remove proteins in an aqueous environment. As no solvents are employed, it also can be used to gauge whether or not proteins factor into phenotypic cell response.

Chan DW, Yung MM, Chan Y-Sang, Xuan Y, Yang H, Xu D, Zhan J-Biao, Chan KK, Ng T-Bun, Ngan HY, MAP30 protein from Momordica charantia is therapeutic and has synergic activity with cisplatin against ovarian cancer in vivo by altering metabolism and inducing ferroptosis, Pharmacological Research (2020), doi: https://doi.org/10.1016/j.phrs.2020.105157

Cross-reference classifiers
Disease: Cancer
Sample Type: Omental conditioned media
Cell Response: Cancer cell lines

Increasing evidence shows that Traditional Chinese Medicine (TCM) has an obvious appeal for cancer treatment, but there is still a lack of scientific investigation of its underlying molecular mechanisms. Bitter melon or bitter gourd (Momordica charantia) is an edible fruit that is commonly consumed, and it is used to cure different diseases in various ancient folk medical practices. The investigators report that a bioactive protein, MAP30, isolated from bitter melon seeds exhibited potent anticancer and anti-chemoresistant effects on ovarian cancer cells. To support the analysis of MAP30 altering glycolysis and lipid metabolism in ovarian cancer cells, the authors state "Nile Red fluorescence of lipid-loaded ES2 (human epithelial ovarian cancer cells) in OCM culture for 48 h. …OCM (Omental conditioned medium) with **Cleanascite™** treatment were used as negative controls.". The authors conclude that natural MAP30 is a non-toxic supplement that may enhance chemotherapeutic outcomes and benefit ovarian cancer patients with peritoneal metastases.



nature

Age-induced accumulation of methylmalonic acid promotes tumour progression

Gomes, A.P., Ilter, D., Low, V. *et al.* Age-induced accumulation of methylmalonic acid promotes tumour progression. *Nature* (2020). https://doi.org/10.1038/s41586-020-2630-0

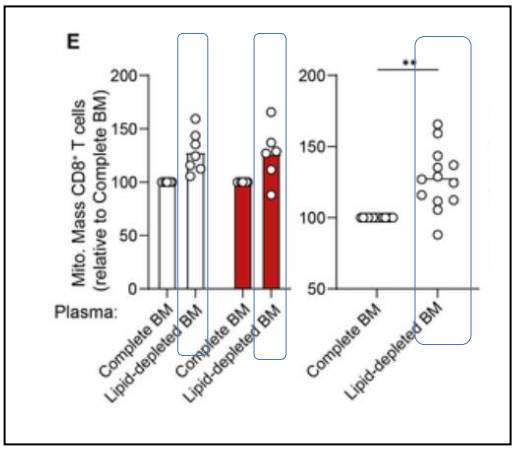
Cross-reference classifiers
Disease: Cancer
Sample Type: Human serum
Cell Response: Cancer cell lines

The risk of cancer and associated mortality increases substantially in humans from the age of 65 years onwards. The authors describe how metabolic alterations that occur with age can produce a systemic environment that favors the progression of tumors. Specifically, that methylmalonic acid (MMA), a by-product of propionate metabolism, is upregulated in the serum of older people and functions as a mediator of tumor progression. To

support this analysis, the authors state "HS (human serum) samples were manipulated to assess the components … that might facilitate entrance of MMA into cells. To delipidate the HS, **Cleanascite** Lipid Removal Reagent (Biotech Support Group) was used according to the manufacturer's protocol …, using a 1:4 volume ratio of **Cleanascite** reagent to sample". The authors conclude that depletion of lipidic structures from serum taken from an older population, resulted in a reduction in total serum MMA levels and was sufficient to abrogate the pro-aggressive phenotype. The data show that MMA, complexed with lipidic structures, is a circulatory factor that contributes to the pro-aggressive effects of ageing in cancer cells and is sufficient to drive tumour progression and aggressiveness. Thus, MMA is a promising therapeutic target for advanced carcinomas.

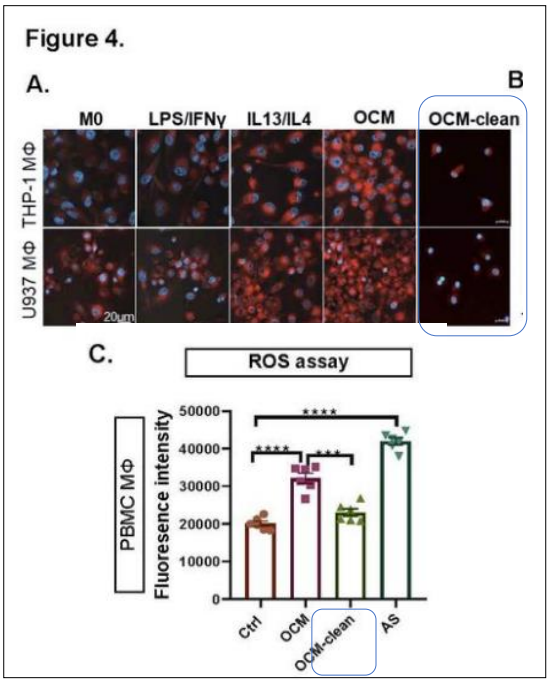
Gudgeon, Bishop, et al. "CD8+ T cell metabolism and function are suppressed by long-chain fatty acid uptake from the bone marrow microenvironment in Multiple Myeloma." (2023).

Cross-reference classifiers
Disease: Cancer
Sample Type: Bone marrow aspirate
Cell Response: T cell activity



Multiple Myeloma (MM) is a plasma cell malignancy that develops in the bone marrow. Function of T lymphocytes is impaired in patients with MM and the bone marrow microenvironment is described as hostile for T cell activity. Precise suppressive mechanisms within the bone marrow microenvironment remain poorly defined. In this study T cell phenotype, function and metabolic activity were analyzed within paired bone marrow aspirate and peripheral blood samples from 72 patients across the spectrum of MM. The bone marrow microenvironment was also modelled in vitro using autologous plasma co-culture systems.

In vitro modelling confirmed that uptake of bone marrow lipids suppresses CD8 + T function, which was impaired in autologous bone marrow plasma, but rescued by both lipid removal and inhibition of lipid peroxidation. The article states "Removal of BM lipids (using **Cleanascite**) increased mitochondrial mass in control and MM BM CD8 + T cells (Fig.), accompanied by restoration of IFN-γ and TNF-α expression.". The article concludes that CD8 + T cells are functionally impaired within the MM bone marrow microenvironment. This is accompanied by decreased mitochondrial mass but elevated uptake of long-chain fatty acids. Blockade of fatty acid transport protein 1 (FATP1) restores CD8 + T cell function in presence of BM lipids and may therefore represent a novel therapeutic target to augment their activity in the bone marrow in MM and improve efficacy of T cell directed therapies.



Chan, David, et al. "Polyunsaturated Fatty Acids Promote Protumoral Macrophage Polarization via a RhoA-YAP1 Signaling Pathway in the Ovarian Cancer Microenvironment." (2022).

Cross-reference classifiers
Disease: Cancer
Sample Type: Omental conditioned medium (OCM)
Cell Response: Tumor-associated macrophages (TAMs)

Tumor-associated macrophages (TAMs) are crucially associated with tumor development and progression; however, it remains unclear how the tumor microenvironment (TME) rewires the metabolic circuits and preferentially induces TAMs to polarize toward a protumoral phenotype. This study reports that polyunsaturated fatty acids (PUFAs) in malignant ascites promote protumoral M2-like TAMs deposition and facilitate peritoneal metastases of epithelial ovarian cancer (EOC). The article states "To selectively remove lipids, **Cleanascite™** Lipid Removal Reagent (Biotech Support Group) was added to the omental conditioned medium (OCM) according to the manufacturer's suggestions. … Intriguingly, the removal of free fatty acids in OCM by the **Cleanascite™** attenuated lipid droplets deposition in M2-like MΦs and OCM-MΦs (Fig. 4A), indicating protumoral M2-like TAMs exhibited higher lipid accumulation and metabolism in the fatty acid-enriched OCM or the malignant ascites."

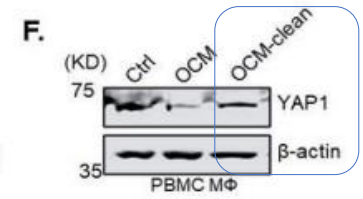
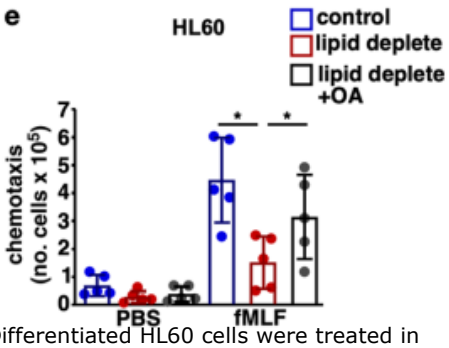


Figure 5. F. MAM led to a reduction in YAP1 activity in PBMC MΦs after 24h treatment. Lipid removal via Cleanascite™ lipid removal reagent in OCM rescued YAP1 activity in PBMC MΦs.

"… Similarly, we demonstrated that the cellular ROS levels in OCM/AS-MΦs derived from PBMC MΦs were significantly upregulated, whereas the addition of **Cleanascite™** mitigated the increased malignant ascites microenvironment (MAM)-mediated ROS level, indicating that the accumulation of UFAs in the MAM is responsible for the enhanced ROS production in TAMs (Fig. 4C). … Here, we further found …removal of lipids by **Cleanascite™** remarkably prevented the reduction of YAP1 in OCM-MΦs (Fig. 5F), implying that the lipids within MAM facilitates protumoral M2-like TAMs polarization through the regulation of RhoA-YAP1 signaling cascade." The article concludes that PUFAs are a key player in promoting tumor-infiltrated TAMs polarization that, in turn, facilitates EOC tumor growth and metastasis.

Pham, Ly, et al. "Neutrophil trafficking to the site of infection requires Cpt1a-dependent fatty acid β-oxidation." *Communications Biology* 5.1 (2022): 1-13.

Cross-reference classifiers
Disease: Infection
Sample Type: Fetal Bovine Serum
Cell Response: Differentiated HL60 cells

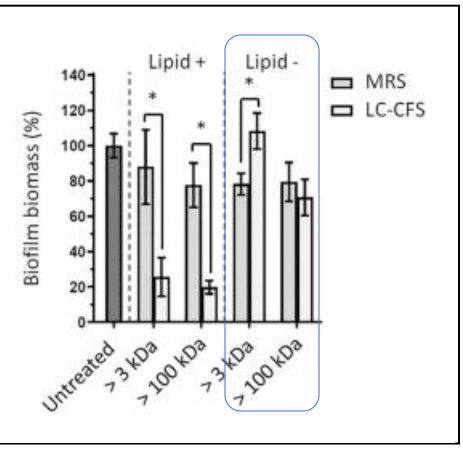


Differentiated HL60 cells were treated in control medium, in medium with lipids depleted, or in medium with lipids depleted supplemented with octanoic acid, and fMLF-induced chemotaxis was assessed using a transwell system (n = 6 per group).

Carnitine palmitoyltransferase 1a (Cpt1a) is considered the rate-limiting enzyme for mitochondrial metabolism of long-chain fatty acids, and Cpt1a deficiency is associated with infant mortality and infection risk. This study was undertaken to test the hypothesis that impairment in Cpt1a-dependent fatty acid oxidation results in increased susceptibility to infection. The article states for lipid depletion "…cells were incubated with RPMI containing 0.5% FBS or with 10% FBS that had been treated with **Cleanascite** (Biotech Support Group) to remove lipids". Observations were made that manipulation of exogenous free fatty acids, either through the depletion of lipids from the culture medium or through the addition of exogenous octanoic acid, alters neutrophil chemotactic functions. The article concludes that susceptibility to pneumonia is associated with blunted neutrophilic responses in mice and humans that result from impaired neutrophil trafficking to the site of infection. Chemotaxis responsible for neutrophil trafficking requires Cpt1a-dependent mitochondrial fatty acid oxidation for amplification of chemoattractant signals. These findings identify Cpt1a as a potential host determinant of infection susceptibility and demonstrate a requirement for mitochondrial fatty acid oxidation in neutrophil biology.

scientific reports

da Silva Barreira, David, et al. "Membrane vesicles released by Lacticaseibacillus casei BL23 inhibit the biofilm formation of Salmonella Enteritidis." *Scientific Reports* 13.1 (2023): 1163.



Cross-reference classifiers
Disease: Biofilms
Sample Type: Membrane vesicles (MVs) (also referred to as extracellular vesicles)
Cell Response: *L. casei* BL23 on *S.* Enteritidis biofilm formation

Biofilms are defined as spatially organized communities of microorganisms embedded in a self-produced matrix of extracellular polymeric substances (EPS), and represent a major concern in the food industry and healthcare. They contain a diversity of elements including polysaccharides, proteins, nucleic acids, lipids and membrane vesicles (MVs) (also referred to as extracellular vesicles), contributing to cellular heterogeneity and resistance to antibiotic treatments. However, the antimicrobial activity of probiotic MVs remains to be studied. The "Generally Regarded as Safe" (GRAS) status of *L. casei* probiotics make them good candidates to investigate antimicrobial activities against pathogens such as *Salmonella enterica*.

The article states "To investigate the effect of *L. casei* BL23 on *S.* Enteritidis biofilm formation, we first quantified the biofilm biomass formed after treatment with several fractions of *L. casei* BL23 cell-free supernatant (LC-CFS). To this end, the LC-CFS and the growth medium of the bacteria (i.e. MRS) were fractionated by size-exclusion ultrafiltration to obtain several fractions ranging from 3 kDa to over 100 kDa".

"Next, we decided to remove all lipids from LC-CFS > 100 and LC-CFS > 3 fractions and their corresponding controls (MRS > 100 and MRS > 3) using a lipid removal reagent…**Cleanascite**; following the manufacturer's instructions." "The antibiofilm activity of the delipidated fractions (Lipid −) was then compared to the initial fractions (Lipid +) by crystal violet staining (Fig.)…we saw a significant increase in biomass formation with the delipidated LC-CFS > 3 fraction compared to the control (MRS > 3) and the untreated fractions." The report showed that the cell-free supernatant (LC-CFS) and the cell lysate of *L. casei* BL23 have a strong antibiofilm activity against *S.* Enteritidis, whereas live cells have no significant effect. Furthermore, it was demonstrated that membrane vesicles (LC-MVs) released by *L. casei* BL23 contribute to the antibiofilm activity of LC-CFS but have no impact on bacterial growth. In addition, it was showed that LC-MVs have a strong antibiofilm effect at the early stage of biofilm formation and no effect on established biofilms of *S.* Enteritidis. Finally, it was demonstrated that proteins associated with LC-MVs are responsible for the antibiofilm effects of the vesicles, and two peptidoglycan hydrolases (PGHs) were found to be involved in vesicle activities.

Jiang, Lifeng, et al. "Akt-Dependent Glycolysis-Driven Lipogenesis Supports Proliferation and Survival of Human Pulmonary Arterial Smooth Muscle Cells in Pulmonary Hypertension." *Frontiers in medicine* 9 (2022).

Cross-reference classifiers
Disease: Pulmonary Arterial Hypertension
Sample Type: Fetal Bovine Serum
Cell Response: Pulmonary arterial vascular smooth muscle cells

Hyper-proliferation of pulmonary arterial vascular smooth muscle cells (PAVSMC) is an important pathological component of pulmonary vascular remodeling in pulmonary arterial hypertension (PAH). Lipogenesis is linked to numerous proliferative diseases, but its role in PAVSMC proliferation in PAH remains to be elucidated. The study aimed to evaluate the status and role of lipogenesis in PAH PAVSMC proliferation and survival. The article states "To achieve the lipid-deprived cell culture condition, cell culture grade fetal bovine serum (FBS) and BSA were delipidated using **Cleanascite™** … Briefly, **Cleanascite™** was added to the FBS or BSA (1:4 volume ratio). The mixture was incubated for four hours at 4°C with gentle shaking and centrifuged at 16,000 *g* for 10 min. Supernatant was collected, then a second dose of **Cleanascite™** Lipid Removal Reagent was added (1:4 volume ratio), and incubation and centrifugation steps were repeated as described above. The supernatants, consisting of lipid depleted FBS or BSA, were then used as cell culture media supplements to prepare lipid-depleted media.". The study found that lipid accumulation detected in PAH PAVSMC was preserved in media deprived from exogenous lipids, indicating that PAH PAVSMC have an ability to generate lipids *de novo.* Importantly, PAH PAVSMC, in contrast to control cells, demonstrated increased growth not only in serum-deprived media, but in lipid-deprived serum-deprived media. The authors conclude that these data demonstrate that human PAH PAVSMC have up-regulated lipogenesis, which is supported in an Akt- and glycolysis-dependent manner and is required for increased proliferation and survival.

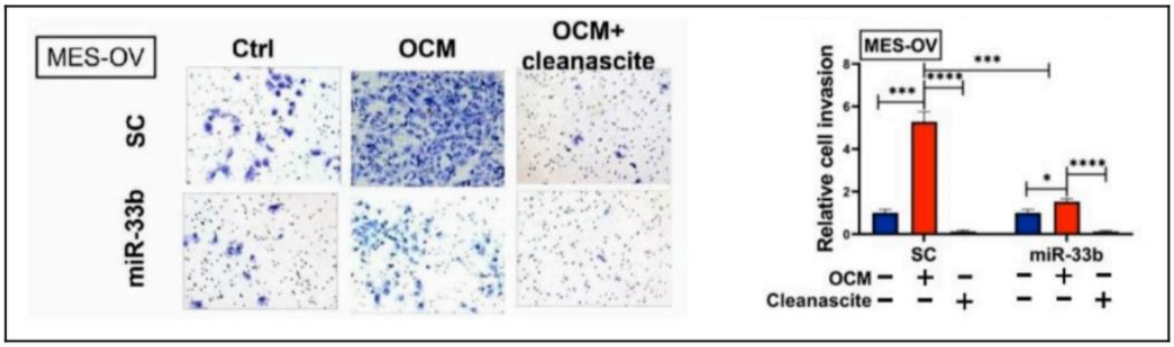
Albakri, Marwah M., et al. "Fatty acids secreted from head and neck cancer induce M2-like Macrophages." *Journal of Leukocyte Biology* (2022).

Cross-reference classifiers
Disease: Cancer
Sample Type: Tumor-conditioned medium
Cell Response: Phenotypic changes in Macrophages

Tumor-infiltrating monocytes can mature into Macrophages that support tumor survival or that display antitumor properties. To explore mechanisms steering Macrophage maturation, the authors assessed the effects of supernatants from squamous cell carcinoma cell lines (FaDu and SCC) on monocyte-derived Macrophage maturation. To assess depletion of fatty acids from tumor supernatants, tumor-conditioned medium was treated with Cleanascite according to the manufacturer's instructions and prior to incubation with monocytes. The article states "Depletion of Fatty acids with Cleanascite from FaDu or SCC supernatants largely reversed the phenotypic changes in Macrophages otherwise observed by incubating monocytes in these supernatants". Macrophages incubated with either palmitic acid or oleic acid developed similar phenotypes as cells incubated in tumor supernatants. The authors conclude that fatty acids are sufficient to mediate monocyte skewing towards M2-like cells.

Wang, Xueyu, et al. "Epigenetic Silencing of miR-33b Promotes Peritoneal Metastases of Ovarian Cancer by Modulating the TAK1/FASN/CPT1A/NF-κB Axis." *Cancers* 13.19 (2021): 4795.

Cross-reference classifiers
Disease: Cancer
Sample Type: Omental conditioned medium (OCM)
Cell Response: Cancer cell lines



The tumor microenvironment is known to influence cancer epigenomics, which plays an essential role in promoting tumor development and metastatic progression. To study this, the researchers used ovarian cancer cells cocultured in omental conditioned medium (OCM), which mimics the ascites microenvironment, and in vivo tumor growth. To examine whether fatty acids in OCM are the main source of energy for tumors, all fatty acids in OCM were first removed by **Cleanascite™** Lipid Removal Reagent. The article states "The omental mixture was subsequently added to the cell culture medium supplemented with 1% FBS for 24 h. OCM was filtered and stored at 4 °C prior to the removal of omentum tissues by centrifugation. To selectively remove lipids and cell debris, **Cleanascite™** … was employed for OCM according to the manufacturer's suggestions." XTT cell viability analysis was performed and showed that the cell growth rate of ES-2 and MES-OV cells was remarkably reduced when cocultured in lipid-depleted OCM. The effective use of **Cleanascite™** helped establish that "both miR-33b overexpression and depletion of fatty acids by **Cleanascite** in OCM significantly impaired ovarian cancer cell migration and invasion." The article concludes that that miR-33b is significantly silenced by DNA hypermethylation in metastatic ovarian cancer cells, to adapt to a lipid-rich microenvironment. Restoration of miR-33b was shown to impair lipid metabolic activities and reduce the oncogenic properties of ovarian cancer cells by negatively regulating the TAK1/FASN/CPT1A/NF-κB pathway, indicating that targeting this signaling cascade may be a molecular therapeutic choice for ovarian cancer metastatic progression.

Flori, E., Mastrofrancesco, A., Mosca, S., Ottaviani, M., Briganti, S., Cardinali, G., Filoni, A., Cameli, N., Zaccarini, M., Zouboulis, C.C., Picardo, M., Sebocytes contribute to melasma onset, ISCIENCE (2022), doi: https://doi.org/10.1016/j.isci.2022.103871.

Cross-reference classifiers
Disease: Melasma
Sample Type: Conditioned medium
Cell Response: Skin cell lines

Melasma is a hyper-pigmentary disorder with photoaging features, whose manifestations appear on specific face areas, rich in sebaceous glands. To verify whether sebocyte-specific lipids could contribute to the observed changes in cell lines, the investigators tested lipid depleted irradiated SZ95 conditioned medium to treat these cells. For this, the BSG product **Cleanascite™** was used. The article states: "**Cleanascite** was added to the collected SZ95 medium in a ratio 1:4 and mixed for 10 min at room temperature by gentle shaking. Following centrifugation (16000g) for 1 min at 4 °C, the supernatant was carefully decanted into a clean collection vial and used for experiments." The authors indicate sebocytes as one of the actors in melasma pathogenesis, inducing prolonged skin cells stimulation, contributing to localized dermal aging and hyperpigmentation.

Chen, Hsin-Yi, et al. "Selective abrogation of S6K2 maps lipid homeostasis as a survival vulnerability in MAPKi-resistant NRASmut melanoma." *bioRxiv* (2021).

Cross-reference classifiers
Disease: Cancer

Sample Type: Fetal bovine serum
Cell Response: Cancer cell lines

The article reports that silencing of the ribosomal protein S6 kinase 2 (S6K2), while preserving the activity of S6K1, perturbs lipid metabolism, enhances fatty acid unsaturation, triggers lipid peroxidation and induces cell death selectively in NRAS-mutant melanoma cells that are resistant to MAPK inhibition. To help identify cell response factors, the article states "Lipid-depleted serum (FBS) was prepared by treating with Cleanascite reagent at a volume ratio of 1:4 per the manufacturer's instructions." The study establishes S6K2 and its effector subnetwork as promising targets for NRAS-mutant melanoma that are resistant to global MAPK pathway inhibitors.

Pointner, Lisa, et al. "Birch pollen induces Toll-like receptor 4-dependent dendritic cell activation favoring T cell responses." *Frontiers in Allergy* (2021): 42.

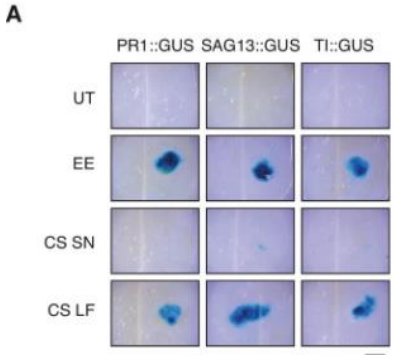
Cross-reference classifiers
Disease: Adaptive Immune Response
Sample Type: Birch pollen extracts
Cell Response: Dendritic cells

This study aimed to examine (i) the importance of Toll-like receptor 4 (TLR4) for dendritic cell (DC) activation by birch pollen extracts (BPE), (ii) the extent of the contribution of BPE-derived lipopolysaccharide (LPS) and other potential TLR4 adjuvant(s) in BPE, and (iii) the relevance of the TLR4-dependent activation of BPE-stimulated DCs in the initiation of an adaptive immune response. The article states "To remove the lipids in BPE, Cleanascite™ was used …according to manufacturer's recommendations … in a ratio 1:1 (v/v). Importantly, "non-specific treatment-associated and cytotoxic effects were ruled out …as neither the protein digestion nor the lipid extraction procedure affected cell activation." These findings suggest that TLR4 is a major pathway by which BPE triggers DC activation that is involved in the initiation of adaptive immune responses.

Stahl, Elia, et al. "Phosphatidylcholines from Pieris brassicae eggs activate an immune response in Arabidopsis." eLife 9 (2020): e60293.

Cross-reference classifiers
Disease: Plant immune response
Sample Type: *P. brassicae* egg extract
Cell Response: *Arabidopsis* cell lines

Recognition of conserved microbial molecules activates immune responses in plants, a process termed pattern-triggered immunity (PTI). Similarly, insect eggs trigger defenses that impede egg development or attract predators, but information on the nature of egg-associated elicitors is scarce. The authors performed an unbiased bioactivity-guided fractionation of eggs of the butterfly *Pieris brassicae* to determine the nature of egg-associated molecular patterns (EAMPs) that induce immune responses in *Arabidopsis*. Preliminary tests indicated that egg-derived defense eliciting compounds are of lipidic nature. To confirm this, the authors stated "we used **Cleanascite** solid-phase aqueous reagent to selectively adsorb lipids from EE (Egg Extract). Application of the lipid-containing phase to …*Arabidopsis* reporter lines triggered strong and localized GUS staining, similar to EE treatment. In contrast, the supernatant containing proteins and other non-lipidic molecules was not active, indicating that defense gene-inducing molecules were restricted to the lipid phase (Figure 1A)". This helped the research in the identification of phosphatidylcholines (PCs) as egg-associated molecular patterns (EAMPs), and illustrated the acute ability of plants to detect conserved immunogenic patterns from their enemies, even from seemingly passive structures such as eggs.



Purification of plant-defense eliciting *P. brassicae* egg lipids.
(A) Expression of defense genes PR1, SAG13, and TI in response to purified *P. brassicae* egg lipids. Purification of egg lipids was conducted using **Cleanascite**. GUS reporter lines were treated with the lipid-free supernatant (CS SN) or the lipid fraction (CS LF) Untreated and egg extract (EE)-treated plants served as controls.

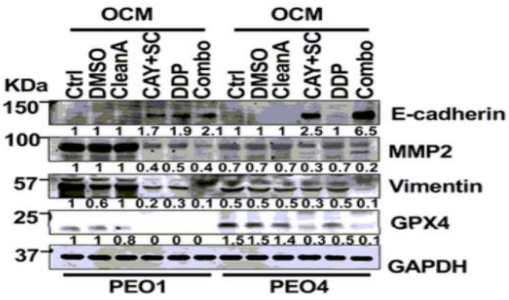
Wang, Haiping, et al. "CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors." *Nature Immunology* (2020): 1-11.

Cross-reference classifiers
Disease: Cancer
Sample Type: Conditioned media
Cell Response: Intratumoral Treg cells

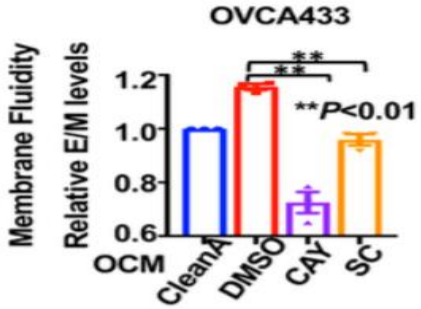
> Depleting regulatory T cells (Treg cells) to counteract immunosuppressive features of the tumor microenvironment (TME) is an attractive strategy for cancer treatment. However, systemic impairment of their suppressive function limits its therapeutic potential. Elucidating approaches that specifically disrupt intratumoral Treg cells is direly needed for cancer immunotherapy. The use of **Cleanascite™** helped demonstrate that intratumoral Treg cells increase lipid metabolism and CD36 expression. The article states "cancer cell-conditioned medium … was treated with **Cleanascite™** reagent (Biotech Support Group) before Treg cell culture at a volume ratio of 1:5 according to the manufacturer's instructions." The study concludes that CD36 targeting elicited additive antitumor responses with anti-programmed cell death protein 1 therapy. The findings uncover the unexplored metabolic adaptation that orchestrates the survival and functions of intratumoral Treg cells, and the therapeutic potential of targeting this pathway for reprogramming the tumor microenvironment.

Yang, X. U. A. N., et al. "SCD1/FADS2 fatty acid desaturases equipoise lipid metabolic activity and redox-driven ferroptosis in ascites-derived ovarian cancer cells." (2021).

Cross-reference classifiers
Disease: Cancer
Sample Type: Ascites-derived OvCa cells
Cell Response" Ovarian cancer cells



OCM-cocultured OVCA433/PEO1/PEO4 cells treated with lipid removal reagent **Cleanascite** (CleanA) for 48 h



Measurement of membrane fluidity evaluated by fluorescence spectroscopy quantification. CleanA reflects **Cleanascite** treatment.

Malignant ascites in peritoneal metastases is a lipid-enriched microenvironment and is frequently involved in the poor prognosis of epithelial ovarian cancer (EOC). However, the detailed mechanisms underlying ovarian cancer (OvCa) cells dictating their lipid metabolic activities in promoting tumor progression remain elusive.

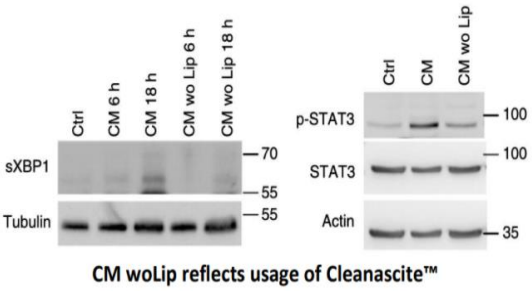
Compared with the negative controls (OCM pretreated with the lipid removal reagent, **Cleanascite**), OvCa cells cocultured in the lipid-enriched OCM showed an increase of 18% in membrane fluidity. The authors conclude inhibition of SCD1/FADS2 directly downregulated GPX4 and the GSH/GSSG ratio, causing disruption of the cellular redox balance and subsequent iron-mediated lipid peroxidation in ascites-derived OvCa cells. Hence, combinational treatment with SCD1/FADS2 inhibitors and cisplatin synergistically repressed tumor cell dissemination, providing a promising chemotherapeutic strategy against EOC platinum resistance and peritoneal metastases.

nature immunology
ARTICLES
https://doi.org/10.1038/s41590-021-01047-4

Di Conza, Giusy, et al. "Tumor-induced reshuffling of lipid composition on the endoplasmic reticulum membrane sustains macrophage survival and pro-tumorigenic activity." *Nature immunology* (2021): 1-13.

Cross-reference classifiers
Disease: Cancer
Sample Type: Culture media
Cell Response" Macrophage cells

Tumor-associated macrophages (TAMs) display pro-tumorigenic phenotypes for supporting tumor progression in response to microenvironmental cues imposed by tumor and stromal cells. However, the underlying mechanisms by which tumor cells instruct TAM behavior remain elusive. The investigators here uncover that tumor-cell-derived glucosylceramide stimulated unconventional endoplasmic reticulum (ER) stress responses by inducing reshuffling of lipid composition and saturation on the ER membrane in macrophages.



CM woLip reflects usage of Cleanascite™

As part of the study, a comparison of culture media (CM) with and without lipids (woLip) was made. The article states "To generate culture media without lipids, …CM was treated with Cleanascite according to the manufacturer's instructions.". The authors uncover the unexpected roles of tumor-cell-produced lipids that simultaneously orchestrate macrophage polarization and survival in tumors via induction of ER stress responses and reveal therapeutic targets for sustaining host antitumor immunity.

Miller-Rhodes, Patrick, and Harris A. Gelbard. The Cell Culture Environment Regulates the Transcription Factor MafB in BV-2 Microglia. " Matters 7.1 (2021): e202010000001.

Cross-reference classifiers
Disease: Central nervous system (CNS)
Sample Type: Conditioned media
Cell Response: BV-2 microglial cells

Microglia experience dramatic molecular and functional changes when transferred from the central nervous system (CNS) to a cell culture environment. This investigation explores lipid dependency as CNS-specific microenvironmental cues, that dictate the gene-regulatory networks specified by master regulator transcription factors such as V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB). To support this analysis, the authors evaluated serum lipid-depletion, stating "Lipids were depleted from FBS using a **Cleanascite** lipid removal agent (Biotech Support Group, cat no. X2555-10). **Cleanascite** reagent was thoroughly resuspended before mixing with FBS at a volume ratio of 1:4 (**Cleanascite**: FBS). The mixture was gently and periodically inverted for 10 min to facilitate lipid binding. The solution was centrifuged at 1,000 x g for 15 min to pellet the removal agent. The resulting supernatant was used for experiments." The report concludes that depletion of lipids, either by serum deprivation or the use of lipid-depleted serum, reduced MafB protein levels in BV-2 microglial cells. In aggregate, the data suggest that serum exposure regulates the transcription factor MafB in BV-2 cells through direct and indirect mechanisms.

Chen, Rain R., et al. "Targeting of lipid metabolism with a metabolic inhibitor cocktail eradicates peritoneal metastases in ovarian cancer cells." *Communications Biology* 2 (2019).

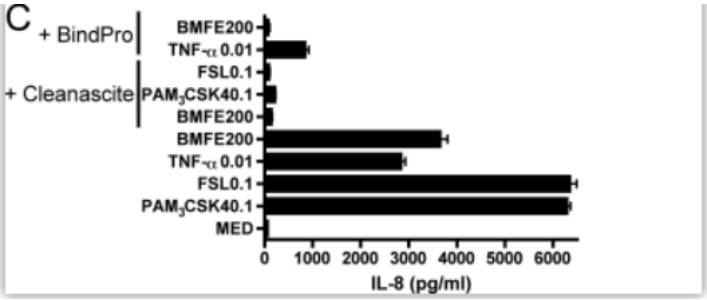
Cross-reference classifiers
Disease: Cancer
Sample Type: Conditioned media
Cell Response: Ovarian cancer cell lines

Ovarian cancer is an intra-abdominal tumor in which the presence of ascites facilitates metastatic dissemination, and is associated with poor prognosis. However, the significance of metabolic alterations in ovarian cancer cells in the ascites microenvironment remains unclear. In this study, the authors investigated whether reprogramming of lipid metabolism in ovarian cancer cells could modulate cell viability and aggressiveness. The article states: "To determine whether fatty acids in OCM are the primary energy source, fatty acids from OCM was first removed by **Cleanascite™** Lipid Removal Reagent... Then, XTT cell proliferation assays showed that the growth rate of ovarian cancer cells was remarkably reduced in cells cultured in **Cleanascite™**-treated OCM (Fig. 2d). Likewise, co-treatment with **Cleanascite™** and OCM significantly attenuated the increased cell migration and invasion capacities of ES-2 and SKOV3 cells (Fig. 2e, f). These findings suggest that the fatty acid-enriched OCM provides as an energy source for supporting tumor growth and aggressiveness of ovarian cancer cells.". The authors conclude that targeting the lipid metabolism signaling axis impedes ovarian cancer peritoneal metastases.

Turner JD, Langley RS, Johnston KL. Wolbachia Lipoprotein Stimulates Innate and Adaptive Immunity through Toll-like Receptors 2 and 6 to Induce Disease Manifestations of Filariasis The Journal of Biological Chemistry.2009;284:22364-22378

Cross-reference classifiers
Disease: Infectious Disease
Sample Type: Soluble *Brugia malayi* female worm extract
Downstream Use Platform: HEK-TLR2 cells

Wolbachia endosymbiotic bacteria have been implicated in the inflammatory pathogenesis of filariasis. Inflammation induced by *Brugia malayi* female worm extract (BMFE) is dependent on Toll-like receptors 2 and 6 (TLR2/6) with only a partial requirement for TLR1. Removal of *Wolbachia*, lipids, or proteins eliminates all inflammatory activity. The article states "To determine if TLR2/6 ligands of *Wolbachia* are lipoproteins, we treated the filarial extracts with **Cleanascite™**, which selectively removes lipids and lipoproteins, or with **BindPro™**, a polymeric protein removal suspension reagent (Biotech Support Group). Both treatments completely ablated (to background levels) HEK-TLR2 cell IL-8 reporter gene activity to BMFE (Fig. 1*C*) thereby showing that the TLR2/6 activity depends on both lipid and protein moieties." The authors conclude that *Wolbachia* lipoproteins drive interferon-dependent $CD4^+$ T cell polarization and antibody switching.



The inflammatory stimuli of BMFE are lipoproteins that primarily signal via TLR2/6. C. triplicate HEK-TLR2 cultures were stimulated with BMFE or control stimuli (doses stated are micrograms/ml) before or following **Cleanascite™** or **BindPro™** treatment. Data plotted are mean IL-8 ± 1S.E.

Lee, Hong-Jai, et al. "Regulatory effect of humoral milieu on the viral DNA and surface antigen expression of hepatitis B virus (HBV) in vitro." *Molecular & Cellular Toxicology* 15.2 (2019): 123-128.

Cross-reference classifiers
Disease: Hepatitis B
Sample Type: Serum
Cell Response: viral DNA and surface antigen expression

The investigations explored if humoral milieu such as serum or culture media, and its constituents, and pH would regulate the viral DNA and surface antigen expression of HBV *in vitro*. Furthermore, lipid removal analysis showed decreased level of HBV DNA and surface antigen expression in human and mouse serum. The article states "To evaluate the lipid exposure status within lipid bilayer, **Cleanascite™** (Biotech Support Group) was added to HBV mixtures in the human serum, mouse serum, or DMEM, and the HBsAg and HBV DNA were evaluated. … we examined the virus-lipid interaction in non-host milieu, and compared the interaction between in host and non-host milieu. The levels of HBsAg and HBV DNA were significantly decreased with lipid removal by **Cleanascite™** in mouse serum rather than human serum". The authors'

concluded that humoral lipid might confer protection to virion against toxicants or hostile interaction with humoral components.

Nguyen, Doan C., et al. "Extracellular vesicles from bone marrow-derived mesenchymal stromal cells support ex vivo survival of human antibody secreting cells." Journal of extracellular vesicles 7.1 (2018): 1463778.

Cross-reference classifiers
Disease: Pan/Methods
Sample Type: Bone marrow-derived mesenchymal stromal cell secretome
Cell Response: Human antibody secreting cells

Bone marrow-derived mesenchymal stromal cells (MSC) have been shown to support human antibody secreting cells (ASC) survival ex vivo. Extracellular vesicles from bone marrow-derived mesenchymal stromal have novel mechanisms of cell-cell communication over short and long distances, but whether the crosstalk between these cell interactions can occur via extracellular vesicles is not known. Thus, the study investigated the role of extracellular vesicles (EVs) in antibody secreting cell survival and IgG secretion. The article states "To understand whether disrupting the lipid plasma membrane that upsets the integrity of the EVs would compromise the survival activity of either the non-irradiated or irradiated secretomes, …We then cultured ASC with conventional media (vehicle), secretome from irradiated MSC, or secretome from irradiated MSC that had been pretreated with the lipid-disrupting agent **Cleanascite™** [28], which is known not to alter protein functionality [29]. **Cleanascite™**-treatment of the secretome dramatically reduced ASC functional survival, … Similar reductions were also noted with the secretome of non-irradiated MSC when treated with **Cleanascite™** … These results demonstrate that lipid-membrane bodies, such as EVs, could mediate important ASC survival factors within the MSC secretome."

Sprenkle, Neil T., et al. "Endoplasmic reticulum stress is transmissible in vitro between cells of the central nervous system." *Journal of Neurochemistry*.

Cross-reference classifiers
Disease: Neurodegenerative
Sample Type: Conditioned media
Cell Response: Stressed astrocytes and neurons

Improper protein folding and trafficking are common pathological events in neurodegenerative diseases that result in the toxic accumulation of misfolded proteins within the lumen of the endoplasmic reticulum (ER). The cell-extrinsic role of sustained unfolded protein response activation under physiological and pathological states in the central nervous system (CNS) remains to be elucidated. The authors studied the characteristics of a mediator secreted by ER stressed astrocytes and neurons. To determine if the mediator was a lipid associated factor, the article states "…100 µl of **Cleanascite™** slurry was added to 1 ml of conditioned medium and incubated at RT with end-over-end mixing for 1 h followed by centrifugation." The authors provided evidence that depletion of lipids from astrocyte conditioned media using **Cleanascite™** abrogated transmission of ER stress. Such evidence helped the authors conclude that ER stressed astrocytes and neurons secrete a molecule(s) with lipid characteristics which regulates both inflammatory and ER stress responses in other astrocytes, neurons, and microglia *in vitro*. These findings provide insight into the cell-nonautonomous influence of ER stress on cells of the central nervous system.

Barrera N, dos Santos Neto PC, Cuadro F, Bosolasco D, Mulet AP, Crispo M, et al. (2018) Impact of delipidated estrous sheep serum supplementation on in vitro maturation, cryotolerance and endoplasmic reticulum stress gene expression of sheep oocytes. PLoS ONE 13(6): e0198742. https://doi.org/10.1371/journal.pone.0198742

Cross-reference classifiers
Disease: Cryosurvival
Sample Type: Estrous sheep serum
Cell Response: Oocyte maturation

High lipid content of oocytes and embryos in domestic animals is one of the well-known factors associated with poor cryosurvival. In this articles, the authors wanted to determine whether the use of delipidated estrous sheep serum during in vitro maturation (IVM) of ovine oocytes reduces the cytoplasmic lipid droplets content and improves embryo development and cryotolerance after vitrification. The article states "Lipid removal from serum was performed by using **Cleanascite™** (Biotech Support Group, NJ, USA) according to the instructions provided by the manufacturer. Unlike other approaches, the protocol described herein for delipidation of estrous sheep serum was effective in decreasing levels of Triglycerides, total Cholesterol, and NEFAs. To our knowledge this is

the first study to use the **Cleanascite™** method to generate estrous sheep serum yielding significantly reduced lipid levels. Subsequent use of the partially delipidated serum as supplemented in IVM media resulted in effective reduction of oocyte lipid content. The advantage of this method over other traditional methods (i.e. chloroform) includes increased feasibility and reduced toxicity and biosafety concerns". Their results demonstrate that although supplementation of IVM medium with delipidated estrous sheep serum reduces the presence of cytoplasmic lipid droplets in oocytes after maturation, oocyte cryotolerance is not improved.

Dean, E. Danielle, et al. "Interrupted glucagon signaling reveals hepatic α cell axis and role for L-glutamine in α cell proliferation." Cell metabolism 25.6 (2017): 1362-1373.

Cross-reference classifiers
Disease: Diabetes
Sample Type: Mouse serum
Cell Response: α cell proliferation

Decreasing glucagon action lowers blood glucose and may be useful therapeutically for diabetes. However, interrupted glucagon signaling leads to α cell proliferation. In this article, the authors wanted to determine which factors affected α cell proliferation. The article states "For lipid removal, whole mouse serum was treated with **Cleanascite™** reagent (Biotech Support Group, Monmouth Junction, NJ) prior to islet culture at a 1:1 ratio according to the vendor's protocol. Lipid removal was validated by HPLC to remove 99% of all phospholipids, cholesterols, and triglycerides….". In testing whether lipids could stimulate α cell proliferation, it was found that serum activity was retained after the removal of >99% of triglycerides, cholesterols, and phospholipids. The authors conclude that amino acids, especially L-glutamine, regulate α cell proliferation and mass via mTOR-dependent nutrient sensing.

Lovászi, M., et al. "Sebum lipids influence macrophage polarization and activation." *British Journal of Dermatology* (2017). doi: 10.1111/bjd.15754.

Cross-reference classifiers
Disease: Cell Biology
Sample Type: Sebocytes
Downstream Use Platform: Immortalized human SZ95 sebocytes

The article's authors report on sebum lipids contributing to the differentiation, polarization and function of macrophages. In order to determine the role of specific lipids, lipid removal was investigated from supernatants of the immortalized human SZ95 sebocytes, as stated, "For lipid depletion of the supernatants **Cleanascite™** lipid clarification reagent (Biotech Support Group, Monmouth Junction, NJ, USA) was used according to the manufacturer's instructions. Lipids; squalene, linoleic acid, oleic acid, palmitic acid and stearic acid (Sigma-Aldrich); were replaced individually subsequent to lipid depletion in a concentration of 150 μM.". The authors concluded a role for sebaceous glands in modulating immune responses via their secreted lipids that are of possible pathologic and therapeutic relevance.

Chan, DW, Mak, SL, Ngan, HYS. The significance of lipid metabolism in peritoneal metastases of ovarian cancer. The 2016 Cold Spring Harbour Asia Conference on Cancer and Metabolism, Suzhou, China, 19-23 September 2016. http://hub.hku.hk/handle/10722/235385

Cross-reference classifiers
Disease: Cancer
Sample Type: Omental explant culture
Downstream Use Platform: Ovarian oncogenic capacities

The authors report that the high lipid content in ascetic fluid provides a huge energy source for ovarian cancer cells in peritoneal dissemination and intraperitoneal tumor colonization. In this study, ovarian cancer cells co-cultured with an omental explant culture system (OCM) or ascetic fluid from ovarian cancer patients exhibited an increase in *in vitro* cell growth, cell migration/invasion through activation of TAK1/NF-kappaB signaling cascade. The abstract states "In contrast, the oncogenic capacities of ovarian cancer cells were impaired when cultured in OCM treated with **Cleanascite™** Lipid Removal Reagent, suggesting that the bioactive lipids in OCM are required for enhanced oncogenic capacities".

Kamtchoua, Thierry, Monica Bologa, Robert Hopfer, David Neveu, Branda Hu, Xiaohua Sheng, Nicolas Corde, Catherine Pouzet, Gloria Zimmerman, and Sanjay Gurunathan. Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults. Vaccine (2012).

Cross-reference classifiers
Disease: Infectious Disease Vaccine
Sample Type: Serum
Downstream Use Platform: toxin-neutralizing antibody titer

> Authors Thierry Kamtchoua et al published an article in the journal Vaccine titled, 'Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults' describing the immunogenicity of pneumococcal single antigen protein vaccine in a phase 1, randomized, placebo controlled dose escalating study. Authors cite **Cleanascite™** from Biotech Support Group for removal of cholesterol from serum. A toxin neutralizing assay with antibodies in sera was developed to neutralize cytotoxicity caused by Ply in Vero cells. An incubated challenge dose of pneumolysin toxin containing serum diluted with or without **Cleanascite™** was developed. The neutralizing titer inhibited the toxin's effect on Vero cells. According to the paper, "Briefly, the toxin-neutralizing antibody titer was determined by incubating a challenge dose of pneumolysin toxin with serial 2-fold dilutions of serum treated with or without **Cleanascite™** (Biotech Support Group) to remove cholesterol, an inhibitor of Ply".

Antunes RF; Brandao C; Maia M; Arosa FA. Red blood cells release factors with growth and survival bioactivities for normal and leukemic T cells. Immunology and Cell Biology.2011;89(1):111-21

Cross-reference classifiers
Disease: Cancer
Sample Type: Red Blood Cell media
Downstream Use Platform: T Cell proliferation

> Red blood cells (RBCs) have been implicated since the early 1970s in the modulation of T cell responses both *in vitro* and *in vivo*. As they can also regulate biological processes of neighboring cells, the authors aim was to show that human red blood cell conditioned media contains bioactive factors that favor proliferation of normal activated T cells and leukemic Jurkat T cells. To define whether factors that favor proliferation were lipid associated, the RBC media was depleted of extracellular vesicles by ultracentrifugation. Then, the article states "For lipid depletion, **Cleanascite™** was added to the RBC-sup in a ratio 1:4 and the mixture incubated first in a rotator at room temperature for 10 min, followed by a further incubation at 4ºC for 30 min, following manufacturer's instructions. Then, the mixture was centrifuged to remove the resin and the RBC-sup collected and concentrated as indicated above before the *in vitro* bioactivity assays". The authors conclude that red blood cells release protein factors (not lipid factors) with the capacity to sustain T-cell growth and survival. Such protein factors may have an unforeseen role in sustaining malignant cell growth and survival *in vivo*.

Cheng AM, Moore EE, Masuno T et al Normal Mesenteric Lymph Blunts the Pulmonary Inflammatory Response to Endotoxin. Journal of Surgical Research.2006;136(S2):166-171

Cross-reference classifiers
Disease: Hemorrhagic shock
Sample Type: Mesenteric lymph
Downstream Use Platform: Primary human pulmonary endothelial cells

> LPS induced ICAM-1 expression decreases by lipoproteins in normal mesenteric lymph(NML) which contain anti-inflammatory factors**. Cleanascite™** was used for delipidation and removal of lipoproteins from primary human pulmonary endothelial cells (HMVECs) incubated with normal mesenteric lymph NML or post-shock mesenteric lymph PSML. ICAM expression was measured after LPS stimulation by flow cytometry. ICAM-1 surface expression was measured by flow cytometry. **Cleanascite™** extracted lipoproteins from NML before incubation and LPS-induced ICAM-1 expression was determined. Researchers concluded that decreased lipoprotein expression after hemorrhagic shock HS increases post-shock mesenteric lymph PSML toxicity from the ischemic gut.

Cho N, Chueh PJ, Kim C et al Monoclonal antibody to a cancer-specific and drug-responsive hydroquinone (NADH) oxidase from the sera of cancer patients. Cancer Immunology, Immunotherapy. 2002;51(3):121-9

Cross-reference classifiers
Disease: Cancer
Sample Type: Serum

Downstream Use Platform: Cancer cell cultures

Scientists prepared monoclonal antibodies to a 34-kDa circulating form of a drug-responsive hydroquinone (NADH) oxidase with a protein disulfide–thiol interchange activity specific to the surface of cancer cells and the sera of cancer patients**. Cleanascite™** was used for delipidation of sera. Epitopes (antibody (mAb) 12.1 and postimmune antisera) inhibited the drug-responsive oxidation of NADH with the sera of cancer patients. Authors concluded both mouse ascites containing mAb 12.1 and postimmune sera (but not preimmune sera) slowed the growth of human cancer cell lines in culture, but did not affect the growth of non-cancerous cell lines.

Patents (Third Party)

United States Patent Application 20170348398 entitled: "COMPOSITIONS AND METHODS FOR DECREASING BLOOD GLUCAGON LEVELS"

Cross-reference classifiers
Disease: Diabetes
Sample Type: Serum
Cell Response: Pancreatic α cell proliferation

The patent discloses compositions and methods for decreasing blood glucagon levels. As disclosed, L-glutamine is a selective stimulator of α-cell proliferation generated when glucagon signaling is interrupted. A method for treating a subject with hyperglucagonemia, e.g., a subject with diabetes, that involves administering to the subject a composition comprising an L-glutamine inhibitor in an amount effective to decrease blood glucagon levels, is disclosed. In an example, pancreatic islets were isolated from male 8-14 week old C57B16/J mice (Jackson Laboratory, ME) and cultured in various media conditions for 3 days. The patent states " For lipid removal, serum was treated with **Cleanascite™** reagent (Biotech Support Group, Monmouth Junction, N.J.) prior to islet culture at a 1:1 ratio according to the vendor's protocol. ". The example supports that increased amino acids, but not lipids and other soluble factors, selectively increased rapamycin-sensitive α-cell proliferation.

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Lipid Removal and Clarification (https://www.biotechsupportgroup.com/Articles.asp?ID=456)

Hemoglobin Removal (https://www.biotechsupportgroup.com/Articles.asp?ID=452)

Sample Prep – Liquid Biopsy (https://www.biotechsupportgroup.com/Articles.asp?ID=457)

Sample Prep – Glyco, Virus, Kinase, Aqueous Protein Crash/Metabolomics (https://www.biotechsupportgroup.com/Articles.asp?ID=453)

Sample Prep – Mass Spectrometry (https://www.biotechsupportgroup.com/Articles.asp?ID=432)

Sample Prep – Genomics (https://www.biotechsupportgroup.com/Articles.asp?ID=455)

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