Organoids As a form of Modern Day Silver Lining In Blood Cancer

***Abstract*—** Blood Cancer-in the shape of carcinogenesis, is worldwide recognized, as a recent time catastrophe. Its unique capability of sustaining its dormancy, vulnerabilities, of drug screening methodologies, and most importantly therapeutic resistance of tumor affected stem cells has transformed blood cancer, as hardly curable. To face this challenge; Organoids are figured out to be a possible solution. From a researcher's point of view organoids are generally 3D structured in (vivo) clusters of stem cell molecules, showcasing bio-active capabilities. However, the lower success rate of organoids, bespeaking its initial stages of preclinical level of studies. In addition, most of these models & their implications just only been limited to in (vivo) principles and various forms of cancer exemplifying; Blood lymphoma. Interestingly, some recent milestones of organoids in different research models on metastasis reflect~~s~~ the glimpses of hopes. At this present study, we have worked on organoids and their possible involvement in blood cancer. We have emphasized on organoid modellings both in (vivo) and in (vitro) cell culture, which are some excellent sources for cell analysis. Presently, we have established a model where a Nano-sized in (vitro-vivo) cell clustering~~'s~~ of organoids with an MRI scanning technique been utilized to build a more precise and useful therapeutic tool. This innovative approach would help us to identify the tumors that ~~won't~~will not respond to any conventional therapies. Also in our studies the organoids have shown~~;~~ active cellular level of immunomodulation, leading to a proper signal transduction. As a consequences, this revolutionary model creates opportunities for a better outcome in terms of diagnostics and therapeutics.

***Key word:* Blood cancer, in (vivo-vitro) models, Organoids, Revolutionary model.**

1. INTRODUCTION

From the preface of the eclipse of an unknown erstwhile to the dawns of the most advanced 21st century, Blood cancer has always been figured out to be an unbridled deterrent against the existence of human souls. Leukemia, Lymphoma, and Myeloma [26] are all of the three different existing forms of blood cancer, reflecting the various levels of its fatality and pathogenicity. Its high~~er~~ percentage of ~~its~~ morbidity resembl~~es~~ ~~ing~~ the atrocious side of this havoc. According to some recent data interpretations, Blood cancer is being primarily termed as~~;~~ responsible for the deaths of almost a single living person within a span of every 9 minutes in USA in 2017[23]. Previously utilized drug therapeutics and treatment modalities such as; Surgery, Chemotherapy, Radiotherapy and recently experimented immune therapeutics showing a class of higher success rate by dwindling the death percentage by almost 70 percentiles. However, they are still unable to eradicate this

apocalypse. The primary analytical reports symbolizing the main obstacles behind the treatment policies of blood cancer are:

• The inability to target and the supreme capability of the resistance of human stem cells against various types of cancerous medications.
•Lack of cancer epigenetics profiling and specificity suggesting the unfortunate aspects of its inability to treat tumor, even within the same origin and similar characteristics.
•Metastasis of cancer tumor cells paving a way for some research output on something effective and advanced, especially in blood cancer.
•The Non-specific nature of cancer symptoms and the problems associated with cancer diagnosis making it harder to treat.

Example: The current imaging tool PET-CT technique is still unable to predict the responses with reliable accuracy and not that much effective towards a more individualized treatment policies, urging on the necessity of innovative therapeutic solutions like; Organoids. That's why this proposed theory surrounding the active responses of organoids as an anti-oncogenic agent, has a huge potential to fulfill. Nevertheless the lower success rate of organoids could be used as an obstruction against this proposed one, but here the issued researchology working on the whole aspect, is completely based on the liabilities of those upwardly discussed processes and an advancement of organoid theorem. Furtherly, the vulnerabilities of 2D cell cultures in terms of-

• The Inability to stimulate the micro-environment and organ specific functions and

• Lacking's of the proper genetic heterogeneity of original tumors. Indicating the soften corner in this route of analysis.

Whereas, the activity of 3D in (vivo-vitro) model featuring the followings:

• The effectiveness in both in (vivo) and in (vitro) counterparts and

• The performance of the assay techniques associated with a purpose to differentiation, diagnosis, and its usefulness in vivo self-proliferation and efficiency in the treatment of individually affected cancer cells [2].

From an additional point of view, MRI scanning techniques could be utilized as a trump card in a similar scenario. This Magnetic Resonance Imaging technique possessing, the ability to add a new dimension to the ongoing procedure has the ability ~~by making~~ to make the diagnosis and prognosis process a far more precise and effective in nature. Therefore, the organoids could easily be available to resolve the missing puzzle.

CHALLENGES ASSOCIATED WITH BLOOD CANCER:

The obstacles following the treatment of various blood cancer are:

- While targeting cancer stem cells

- Drug resistance properties of stem cells

- Lack of cancer epigenetic profiling & specificity of existing Epi-drugs.

- Association with cancer diagnosis makes it difficult to treat.

- Unavailability of effective biomarkers in blood cancer.

- Limitations of conventional chemotherapeutic agents.

- Metastasis ~~possessing~~ posing a huge obstacle to the treatment of cancer.

MECHANISM OF BLOOD CANCER:

The stem cells originating from the bone marrow leading to the development of Hematopoiesis. Usually, stem cell molecules are constantly divided to produce a new cell. Whereas, in blood cancer it may sometimes go through a passage of unnatural cell division, anemia or the signal transduction pathway gets severely hampered. ~~AS~~ As a result, the differentiation, transduction, and repair mechanism gets completely damaged, as well as the cell proliferation process.

The greatest armory of these models is just not only to aid us to handle the metabolic changes [2], but also to help us to express the genes. As a consequence~~s~~, **Normal progenitor cells leading to repair and regeneration after the possible occurrence of damages.**

RESEARCH PROCEDURE:

In recent times, the success of both in (vivo) & in vitro organoid cell culture & its wonderful supremacy, while showing mimicry, provides ~~ing~~ the characteristics of heterogeneity [2].

CULTURE SYSTEM OF BLOOD CANCER:

This proposed research model is composed of the following components:

Table 01:

Matrigel Matrix, ECM extract, Advanced DMEM/F12, Gluta Max, HEPES, Noggin, R-Spondin-1, Nicotinamide, A-83-01, Y27632, Gremlin 1, Darbepoetin-alpha, Peginesatide, Romiplostim, WNT pathway inhibitor, Hedgehog pathway inhibitor, Farnesyl transferase inhibitor, Aurora A kinase inhibitor, Histone deacetylase, HSP90, Proteasome inhibitors, Nicotinamide.

It is to be noted that here the existence of ECM substituents is the differentiating constituents between 2D & 3D organoid cell culture[2], where the advanced DMEM/F12 is being utilized as the ideal cell culture media.

Table 02 [13]:

NAME OF THE COMPONNETS OF ADVANCED DMEM/F12:
Glucose
Non-essential Amino Acids
Sodium Pyruvate
Phenol Red

WHY ADVANCED DMEM/F-12 IS UNIQUE:

The reasons to be bolded behind the usage of Advanced DMEM/F-12 are:

• Inexistence of L-glutamine

• ~~There isn't any use of~~ HEPES are not used

• Reduced (FBS) supplementation compared to classics, where reduction occurred by almost (50-60) percentiles [13]

GENERAL OVERVIEW:

Self-developing capability among inherently affected stem cells is a renowned assumption among scientists and has initiated researchers to develop a 3D in (vivo-vitro) cell culture models from primary tissues of bone marrow [2]. Both in (vivo-vitro) models of organoids **representing a more reliable and idealistic response compared to usual cell lines, outlasting recapitulation and manipulation capacity [2].**

WHY NANO-MRI SCANNER:

MRI Scanner is an ideal media to diagnose. Magnetic Resonance Imaging technique uses strong magnetic field gradients and in here, The Nano-ranged wave technology to generate the in (vivo) images of the human body on different slices like; Sagittal, Axial, Limbic to get an ideal diagnosing outcome.

It is~~'s~~ advantageous to use an MRI scanner, as it doesn't have any ionizing radiation technology leading to toxicity. Before going through the MRI scanning process, the subject is ~~being~~ injected ~~by~~ with the dye. Aftermath, Nano ranged estimation aids us to observe and diagnose.

The greatest asset of this type of MRI scanners is the ability to get a gradual improvisation, as the more

Advanced generation reflects on the shorter passage of scanning period.

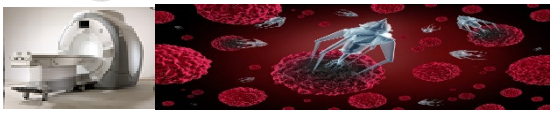
Though it usually takes around (30-60) minutes [14] to make a complete scan, here it has taken a figure somewhere close to (10-15) minutes.

[**NOTE:** **The ideal 3D organoid cell culture having Lamnin riched Matrigel, Growth factors & small cell inhibitors][2]**

In additional sense,

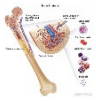
- It would aid the diagnosis quite accurately.
- Greater application of the media.
- It helps in the 3D culture of organoids

(IN VITRO MODEL)

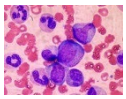


MRI SCANNER [20] **NANOTECHNOLOGY [24]**

DIAGNOSIS [17]



BONE MARROW [25]



STEM CELL [30]

(MINCE)

SMALL FRAGMENTS

(COLLAGENASE TYPE 2 & DIGESTION)

TUMOR STEM CELLS [31]

(ADMEM/F12)

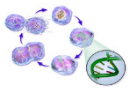
(ENDOSTEAL MATRIX)(FIBRINOCETIN/Cl)

Matrigel +Organoid

Centrifugation

CENTRIFUGED SAMPLE [18]

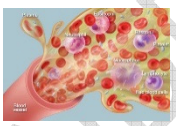
(ISOLATED STEM CELL)



TUMOR STEM CELL [31]

INCUBATION[19]

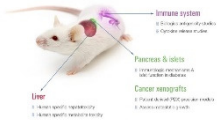
BLOOD SAMPLE [19]



HUMAN BLOOD SAMPLE [32]

Matrigel +Organoid

INJECTION [21]



IN VIVO (RAT MODEL) [27]

Fig. 1: Flow chart for in (vivo-vitro) establishment of Blood Cancer.

IN (VIVO) SCENARIO:

Transgenic mice models ~~is~~ are implemented to resume the experiment in (vivo) analytical condition. Here, the mutated genes of human blood cancer are~~is~~ induced to the growth of blood cancer affected cells. MRI analytical technique is being widely designed for the observational studies.

IN (VITRO) SCENARIO:

The economically balanced, genetically manipulated, and flexibly molded in (vitro) model shows a series of active phenotypic responses. Proving its worth as a recognized assay.

Enzymatic expression in blood cancer is a good option to target. That's why the inhibitors of those channels and their enzymatic activities of the protein level inhibitors been activated. Utilization of Nano wavelength for the purpose of analyzing the targets to establish a proper study model, possessing a superior accuracy and greater efficiency to detect deep lying tumors with relatively ease.

TABLE 03:

GROWTH FACTORS & INHIBITORS APPLIED IN ORGANOID CELL CULTURE

NAME	FUNCTION	STRUCTURE/SOURCE/ COMPONENTS
GREMLIN 1	**Inhibition of predominant BMP2 & BMP4 in limb buds allows**	**•Embryonic fibroblast •Furin like domain •184 Amino acid glycoprotein**

	thetranscriptional upregulation of FGF'S & SHH ligands.	
R-SPONDIN-01	Facilitation of the growth of metastasis[2]	•Chromosome •2cysteine ring •1 thrombospondin type 1 domain
NOGGIN	Promotion of bone metastasis of some cancers & association with tumorigenesis of primary bone malignancies [2]	•HGNC:HGNC:7866
AURORA A KINASE INHIBITOR	•Regulation of serine/threo-nine kinases •Anti-cancer agents	encoding aurora A,B, & C.
FARNESYL TRANSFERASE INHIBITOR	A preventive function	A 4 Amino acid sequence at the carboxyl terminus of a RaS. (48KDa & 46KDa)
HISTONE DEACETYLASE INHIBITOR	Inhibition of histone deacetylase	2classes; HDAC & HDI
PROTEASOME INHIBITOR	Blocks proteasomes	Proteolytic site on the Eukary-otic cells
Wnt PATHWAY INHIBITOR	Promotion of cancer & progression of it[42]	•WNT ligand or receptors •3signaling pathways: canonical, non-canonical planar cell pola rity, non-canonical WNT/calc ium
FLT3	•Formation of fms regulated tyrosine kinase 3 •Signal transduction [28]	HGNC:HGNC:3765[28]
A-83-01	• A transforming growth factor beta inhibitor suppresses the proliferation of organoids [2]	•C25H19N9S • <u>HHI:</u> Results of aberrant compone nt of the Hedgehog signaling pathways. •3different classes; Shh, GLI, SMO [29]
DARBEPOETIN	Stimulates	C815H1317N233O241S5

ALPHA	**•erythropoie-Sis •Anemia**	
PEGINESATIDE	**Stimulates •Anemia •It mimics the structure of Erythropoietin & promotes the RBC development**	**C231H350N62O58S6[C2H4O]n**
ROMIPLOSTIM	**A hormone that regulates platelet production**	**•C2634H4080N722O790S18[47] •Analogue of thrombopoietin**
NICOTINAMIDE	**A Vitamin PP Is a nutrient required for long term organoid culture**	**•C6H6N2O[48] •Nicotinic acid or 3cyanopyridine**
Y27632	**•Inhibition of Rho kinase[2] •Improves culture[2]**	**C14H21N3O**
HEDGEHOG PATHWAY INHIBITOR	**•Inhibites the Growth of cell[•Activates tissue repairmen and cell proliferation**	**•3FDA approved inhibitors: Vismodegib, Erismodegib, Smoothened •It's a kind of glycoproteins**
MATRIGEL INHIBITOR	**• Mimicry in vivo 2D & 3D environments •Improvement of the differentiation of both normal and transformed anchorage dependent epithelial cells**	**Sarcoma cells**
HSP 90 INHIBITOR	**•Inhibits collagen I & ii • Inhibits Matrix metalloprot-Eanase-3 to Reduce cell Metastasis**	**3 types of Natural product geldanamycin (C29H40N2O9), radicicol(C18H17ClO6), 17AAG(C31H43N3O8)**

CLASSIFICATION OF BLOOD CANCER:

Blood cancer can easily be divided into the following way:

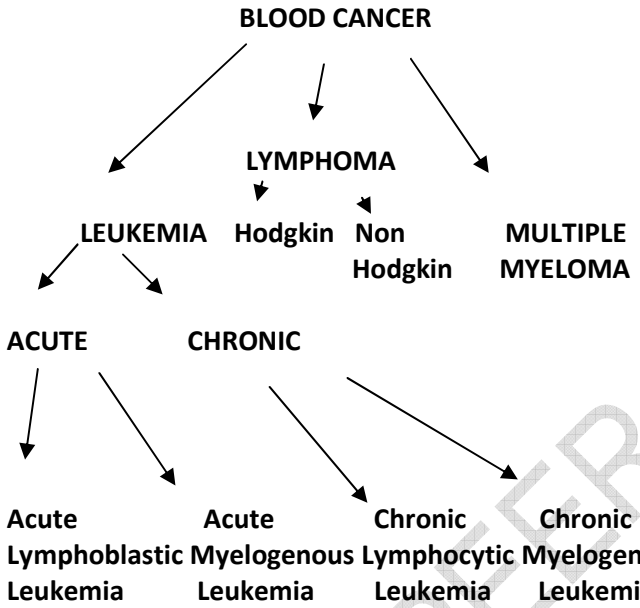


Figure 2: Classification of blood cancer

ACUTE LYMPHOBLASTIC LEUKEMIA:

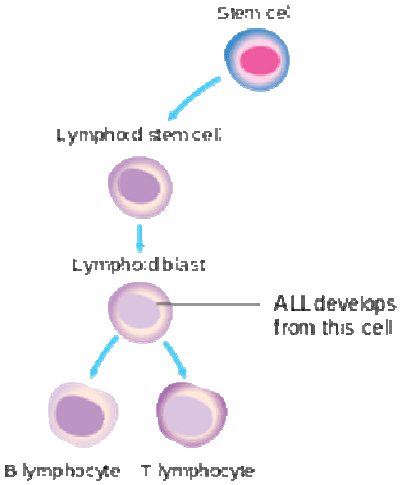


Figure 02 : Acute Lymphoblastic Leukemia [6]

MECHANISM:

SINGLE CELL LYMPHOBLAST → **MUTATIONS TO RELATED GENES**

↓

CEASATION OF THE CELL DEVELOPMENT

↓

CELL PROLIFERATION

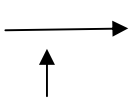
↓

ACUTE LYMPHOBLASTIC LEUKEMIA

MECHANISM OF INHIBITORS:

- **Inhibits tyrosine kinase inhibitors.**

•**Activates proteins by signal transduction cascades.**



[NOTE: Tyrosine kinase inhibitors ability to deprive Tyrosine kinase to access HSP 90]

ACUTE MYELOGENOUS LEUKEMIA:

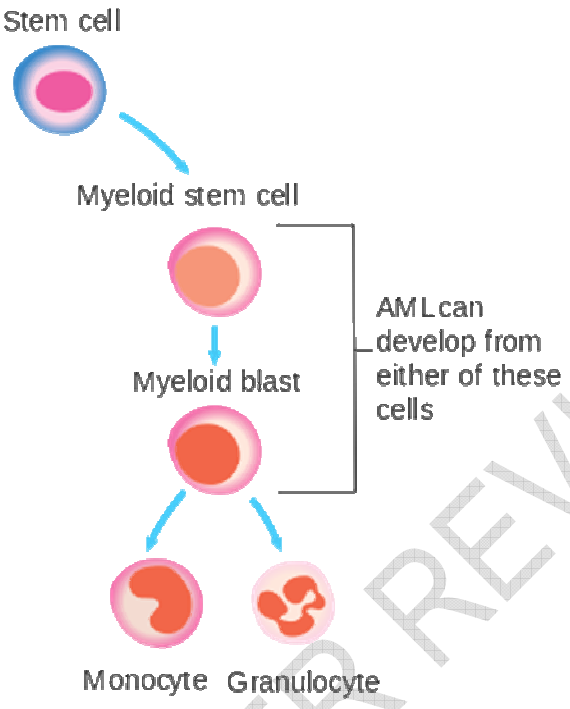


Figure03: Acute Myelogenous Leukemia [7]

MECHANISM:

ABNORMAL CELL DIVISION **GENETIC CHANGES**

ABNORMAL CELL GROWTH IN THE BONE MARROW

GATA 2 TRANSCRIPTION FACTOR DEFECIENCY INDUCES Acute Myelogenous Leukemia

INHIBITORS:

- **Histone deacetylase.**
- **Tyrosine kinase inhibitors.**

CHRONIC LYMPHOCYTIC LEUKEMIA:

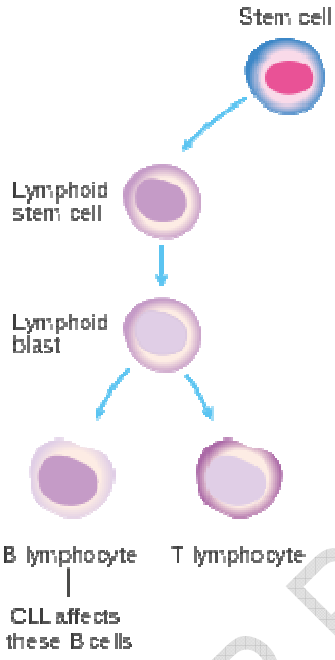


Figure04: Chronic Lymphocytic Leukemia [8]

Chronic Cancer results as the bone marrow produces a handful number of ~~amount of~~ lymphocytes.

REASONS:

- **Genetic mutations**
- **Epigenetic changes**

MECHANISM:

HOMEOSTATIC IMBALANCE

▼

OVER-EXPRESSION OF ANTI-APOPTIC GENES

+

PROTO-ONCOGENE MYC

↓

AGGRESSIVE BETA CELL MALIGNANCIES

↓

(INHIBITOR)

THE DECREASE OF THE PROSPENSITY OF THESE CELLS FOR APOPTOSIS

INHIBITORS:

- **BCl-2 inhibitor**
- **Bruton's tyrosine kinase inhibitor**
- **Phosphoinositide-3-kinase inhibitor**

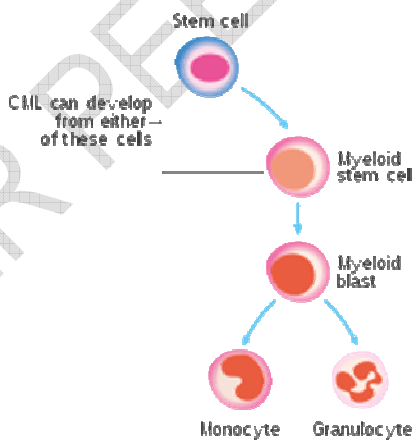


Figure05: Chronic Myelogenous Leukemia [9]

MECHANISM:

DEVELOPMENT & UN-REGULATORY GROWTH OF MYELOID BONE MARROW CELLS

↓

INHIBITORS ⟶

LEUKOCYTOSIS

INHIBITORS:

Tyrosine kinase inhibitors [9]

LYMPHOMA:

2 types of Lymphoma. Hodgkin & Non-Hodgkin lymphoma.

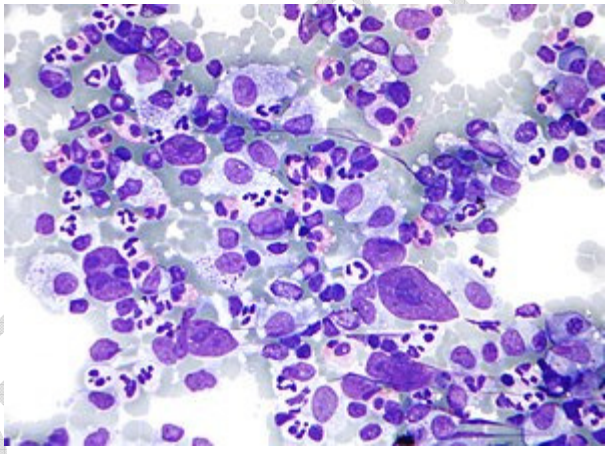


Figure06: Hodgkin Lymphoma [10]

HODGKIN LYMPHOMA:

- **Lack of CD surface antigens results in Hodgkin lymphoma [11].**

•**MOPP was initially used to treat Hodgkin lymphoma.**

NON-HODGKIN LYMPHOMA:

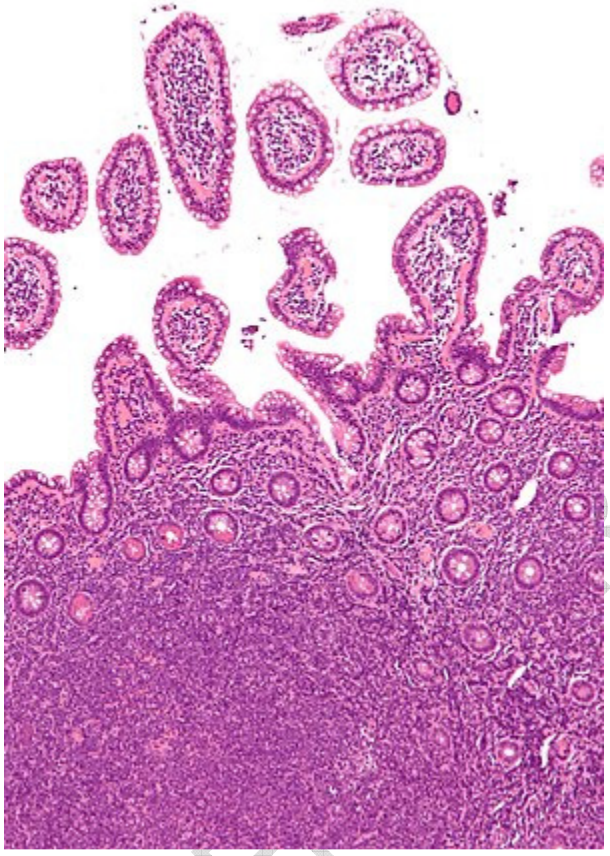


Figure07: Non-Hodgkin Lymphoma [11]

INHIBITORS:

Rituximab works against CD20, but not active against Hodgkin Lymphoma.

MULTIPLE MYELOMA:

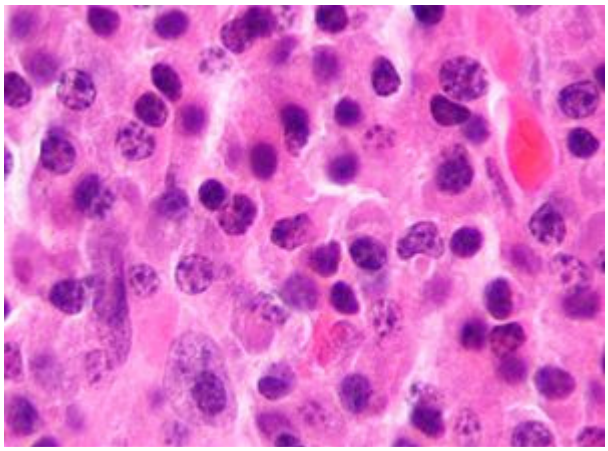


Figure08: Multiple Myeloma [12]

MECHANISM:

NORMAL RBC PRODUCTION

(CYTOKINES) → HEMATOPOIESIS

Inhibition

MULTIPLE Myeloma

INIBITIORS:

Bisphosphonates

INHIBITORS →

BONE RESORPTION BY REDUCING THE NUMBER & ACTIVITY OF OSTEOCLASTS

LIMITATIONS OF THE THEOREM:

The vulnerabilities of the current proposal are:

- **The Organoids are imperfect for reproductions [2].**
- **It can affects the therapeutic potential.**
- **Some organoid lines cannot be expanded, in case of long Term prospects [2].**
- **Cancer organoids tends to grow slowly [2].**
- **It just a research proposal, which requires to be worked gradually on the progression of advancement.**
- **In this study there isn't any discussion about the Acute Monocytic leukemia and its possible treatment.**

GENERAL ORGANOIDS WORKING DIAGRAM (IN VIVO):

MECHANISM 01:

ORGANOIDS

▼

Acquires relative genetic &epi-genetic Information's about tumor cells

↓

Generation of tumor reactive T-cells

↓

Tumor Killing

MECHANISM 02:

ORGANOIDS → Slows the infiltration through The Exchange of biomaterials/chemicals

↓

Positive effects on drug responses used in blood cancer

GENERAL ORGANOIDS WORKING DIAGRAM (IN VITRO):

MECHANISM 01:

ORGANOIDS ⟶ Genetic Mutations

MECHASIM 02:

ORGANOIDS ⟶ CELL GROWTH

MECHANIM 03:

Figuring the tumor immunity ⟶ Signal transduction

ORGANOIDS

MECHANISM 04:

ORGANOIDS ⟶ Improves cell monitoring

↓

Better drug action

CONCLUSION:

The efficiency of organoid molecules and its prowess towards various types of blood cancer, showing a significant active role to establish an ideal in (vivo-vitro) models. However, the upwardly discussed results and their experiments bespeaking the possible crucial interventions against the cell growths. The role of a 3D cell cultured organoid technology is very useful in terms of possible blockage to the affected tumor stem cells and aiding `the transduction mechanism of the normal cell molecules.

Here, The Nano-ranged MRI technology not just only been restricted to its application towards cancerous medications and diagnosis, but also has the power to instrument furtherly to cease the whole associated challenges by providing a possible greater diagnosis and innovative regenerative solutions for the future novel anti-blood cancer therapy.

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