Multi-Phase Based Signature and Cancer Management: An Insight in Embryo, Brain Tumor, Leukemia, and Von Hippel Lindau Syndrome

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Abstract: It has initiated the multi-phase (MPh) phenomen through the cell cycle machinery, and is an icon related to the novel cell cycle M.Ph, characteristics in variety of neoplastic disorders, including cancer.

Cell cycle governs the whole cell based machinery, initiation, progression, and therapeutic platforms in the neoplastic disorders. Differentiation between benign and malignant cells relies on the functional data within the cell cycle domain. Cancer initiation, progressive manner and therapeutic sphere are also dictated by cell cycle.

Proliferation and growth play key role in initiation and develoment of tumors, including benign and malignant cells. The cell cycle's checkpoints are restrictively under the cell cycle control. As the matter of fact, it was trusted that there is no return through the routine cell cycling and it was characterized as an everlasting forward cycling manner. But this fact has been revolutionized through the presence of Mosaic multi-phase (M.Ph) based at single cell level. The programmed checkpoints control the transition of phases through the related barriers. Therefore, balancing the carcinogenic processes is capable to control progression, facilitate and guarantee the most effective and personalized/ target based therapy

The multi-phase based strategy has been also performed in the circulating embryonic, fetal chorionic sample (CVS), chronic myeloblastic leukemia and Von Hippel lindau (VHL) syndrome,

Conclusively, early predictive/prognostic value of MPh provides a reliable, personalized diagnostic and diverse target-based therapeutic platforms in different medical complications.

Keywords: Cell cycle, Individualized, Multi-phase, Early detection, Embryo/fetal, Brain tumor (BT), Chronic Myeloblastic leukemia (CML), Von Hippel lindau (VHL), oocyte, Chorionic villus sample, Non-Invasive, Hypothesis, Evolution, Therapy.

INTRODUCTION

Multi-phase cell cycle signature has unmasked the early and novel fundamental roots in the neoplastic disorders, including solid tumors and leukemias. Key role of single cell based signature and functional characteristics provide an innovative spectrum at embryonic, fetal, post- birth and all through the target individuals' life with family history of neoplastic disorders, and/or being disposed to the in micro- and macro-environmental hazards; and the patients affected with variety of neoplastic disorders including cancer.

An initial report on the *Mosaic Phases through the cell cycle has been published in the primary breast cancer patients* [1]. The hidden events including the predisposing genetic factor, including D1853N polymorphism of Ataia Telangeictasia Mutated gene (ATM), play an influential key role for being affected with neoplastic disorders through the pedigrees [2] The

check points control the evolutionary conversion through the related phases and barriers [1,3]. Furthermore, complementary functions including proliferation, growth, or apoptosis; cyclin dependent kinases, cyclins and retinoblastoma (Rb1) gene enable the transitional processes through growths and synthesis barriers [4,5].

It was trusted that transition of specific phases through the related barriers requires programmed check points [3]. Besides, the requirements for a successful G1/S transition are facilitated by the sequential events including proliferation, growth, or apoptosis; cyclin dependent kinases, cyclins and retinoblastoma (Rb1) gene [3,4]. CCND1 polymorphism rs614367 regulates the expression of CCND1 gene in breast cancer (BC) [6].

The sequential events within the cells include the followings:

1)   At early G1, the complex of cyclin D/CDK-4 or 6 is responsible for the initial phosphorylation of pRb in G1;

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2)   Cyclin E binds and activates CDK2, to phosphorylate pRb in late G1;

3)   In S-phase, the complex of cyclin A/CDK2 leads to further functions; and

4)   Cyclin B1/CDK1 complex facilitate transition through G2-M phases [7-9] and

5)   Evolution and heterogeneity are detectable in metaphase or in interphase by classical and molecular cytogenetic investigation [10].

Von hippel lindau (VHL) is an autosomal dominant genetic disease. It is characterized by almost 13 disorders including hemangioblastomas of the brain, spinal cord and Retina, renal cysts and clear renal cell carcinoma, pheochromocytoma and paraganglioma; pancreatic cysts and neuroendocrine tumors; endolymphatic sac tumors; neuroendocrine tumors; and epididymal and broad ligament cystadenomas [11].

The motivation of present research includes:

1)   To explore the multi-phase phenomena involved in cell cycle domain in three target diverse groups of diseases including the circulating embryonic, fetal chorionic sample (CVS), Chronic myeloblastic leukemia (CML) and Von Hippel lindau (VHL) syndrome (Figure **1**),

2)   possible application of target- based diagnosis to unmask the novel personalized/Predisposing/Predictive/ Prognostic/Preventive (5xP) markers), as a pentatonic –formulated- periodic profile based on the combined phases of the cell cycle-architecture at the single cell level.

3)   to apply an innovative cell cycle MPh- based strategic therapy through The Complex/*Mosaic Phases* (CMPs). However, cell cycle is capable to shape the initiation, progression and therapeutic approaches of neoplasms, including benign and cancer.

MATERIALS AND METHODS

The key role and central position of cell cycle and the performance of analytical process at interphase has been previously provided in an earlier publication [1] Exploration and analytical process are performed according to the screening of multi- phases (MPs) including G1 as single chromatid -, fragmented form in S- and G2 based on the presence of two chromatids.

Fluorescence In Situ Hybridization (FISH), immunofluorescence (IF) was performed according to the standard protocol by exploration of the protein expression, and re-confirmed by flow cytometry (FC). Status of MPs was detected, based on the updated protocol [1].

Sample and Materials

The evolutionary based ranking strategy considered and included embryonic (Em) sample at first week of gestation, and chorionic villus sample (CVS) at 8th week, through the dual sampling of the maternal blood.. Brain tumor and peripheral blood samples from two groups of patients affected with brain tumor (BT), and Von hippel Lindau (VHL) were delivered to trace circulating brain tumor cells (CBTCs) and for circulating VHL-neural cells (CNCs). Furthermore, a prone pedigree including a proband.

Methods and Sample Selection

Fluorescent In Situ Hybridization (FISH) and Protein Expression (PE)

The applied new methodology and techniques at cell cycle level, details of FISH and PE, as the final functional step are available in the initial publication [1].

Cyclin D1: 1) is encoded by CCND1 gene amplification, 2) has been traced in variety of tumors tumors; 3) plays a prominent role in the G1 phase, through the progressive process of the target cells by joining with cyclin-dependent kinases including CDK4 and CDK6.

For highlighting the prognostic and predictive roles of CCND1, C-MYC, and FGFR1 amplification, and/or expression precise and consistent analytical procedures and techniques are required; however, details of applied procedures are available [1].

Regarding the characterization of cell cycle phases, the CMP is categorized as Multi-phases cyclic pattern, multi-programmed-potential, and the multi-targeted therapeutics candidate.

Ratio values was calculated based on the frequency of *MPs* between chromosomes- 1/1, 3/3 and 1/3 by considering the variables A-L, between different complexes of dual and triangle cell cycle phases. Distribution of the PE was explored according to the expression intensity including its lacking, moderate and high levels.

RESULTS AND DISCUSSION

The Novel Multi Phase-Based Cell Cycle Platform at Single Cell Level, Translated the Personalized Therapeutic Strategy, Based on the Cell Cycle Phases in Cancer Patients

Nature of the numeric and functional alterations in cancer is the major intersection for therapy.

The novel cell cycle hypothesis (CCH) highlights the mosaic basis of dual and/or multi-phases, as minor clones at single cell level in the breast cancer (BC) patients are escorted by the normal cell population. Such mosaicism provided an archetypal and unique diagnostic and therapeutic model, by applying different mosaic patterns (*MPs)* as well as "G1/S, S/G2 and G1/S/G2, and normal phases (G1, S, G2)" at the single cell level.

Diagnosis is based on the mode of signal copy numbers (SCN) and the related PE. Interestingly MPs were also unmasked in patients with chronic myelogenous leukemia, other solid neoplasms including brain tumor and in patients with Von Hippel Lindau (VHL) complications.

Finally, the predisposing/predictive/prognostic/ preventive square provides an innovative CDKs inhibitor-based therapy in BC and other neoplastic disorders.

Personalized base cancer therapy is the confusing procedure and requires pedigree based data, personalized evolutionary based information, including molecular and functional, at both genomic and somatic single cell level**.** The target territories comprise of cell cycle phases, proteins, telomere length, telomerase, sub-telomere, and Epigenetics. The aim is directing the cell cycle fundamental forces back to normal, by:

1)   Applying personalized, single cell-based approach, at molecular functional level, pedigree analysis, and micro-/macro-environmental factors including nutrition.

2)   carrying out satisfactory high single cell enumeration based on the FISH and protein expression assays;

3)   Decoding the required dosage and combined therapeutic regimens accordingly,

4)   Unmasking the cell cycle combined (mosaic) phases including different Cyclins; and

5)   Bilateral cooperation between Pharmacology, Medicine, and Cancer Genetics/cell biology.

Let's Combine the Evolutionary Based Strategy by Translating the Personalized Data at Single Cell Level (Molecular/ Functional/ Informative), and Pedigree-Based Level to the Personalized Therapy

Chronic Myeloblastic Leukemia (CML)

Chromosome study was performed in a patient affected with chronic myelogenous leukemia (CML) which had transformed to multiple myeloma. This patient had more than 69% of infiltrating myeloma cells in her bone marrow and Philadelphia chromosome was detected in 18 out of 42 analysed cells.. However, the probable presence of some myeloma cells with classic Philadelphia-positive chromosome could be proposed. CML is reported as a stem cell derived neoplasm, and sporadically associated with lymphoproliferative disorders. Few reports revealed an association of CML
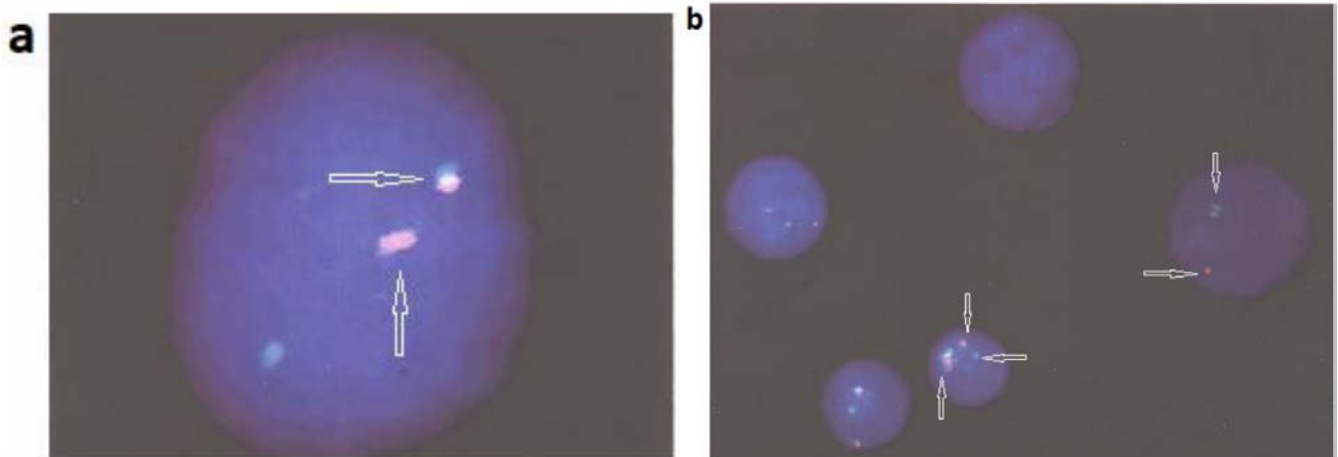

Figure 1: Dual phase in bone marrow cells of a patient with chronic myelogeneous leukemia (CML) with translocation between ABL and BCR genes on chromosome C9 and 22, and dual phase pattern.

with multiple myeloma. Now a confirmed CML patient had transformed into a full-blown multiple myeloma [11]. The mosaic pattern, including an apparently normal clone, accompanied by an abnormal cells, harboring a translocation between q-arms of chromosomes 9 and 22 is provided (Figure **1**).

The mosaic pattern, including an apparently normal clone, accompanied by an abnormal clone with t (9;22) cells which are conjugated with ABL and BCR.

Nuclei containing hybridized signal, with dual color in each nucleus, reflect the occurrence of translocation between chromosomes 9q arm and 22q arm. Mosaic pattern is diagnosed which provide diverse prediction.

a.   Cell at the top/right side: carries t(9;22) in G1 phase, and the cell at bellow/middle, as in G2 phase; and bellow/left side cell is normal and in G1.

b.   The cell at right side contain G1(down:in red) and G2 (up in blue), and another cell with arrow bellow the image, presents chr. 9 and 22, both at G1 (in red and green color)- and another in S-phase (with translocation between chromosomes 9 and 22). Magnification:x1000.

Von Hippel Lindau (VHL)

VHL is characterized as an autosomal dominant genetic disease. It is characterized with hemangioblastomas of the brain, spinal cord and

Retina, renal cysts and clear renal cell carcinoma, pheochromocytoma and paraganglioma; pancreatic cysts and neuroendocrine tumors; endolymphatic sac tumors; and neuroendocrine tumors; and epididymal and broad ligament cystadenomas [12]. The mosaic multi-phase based novel characteristics was also unmasked and will be discussed.

The proband was affected with VHL. His father, mother, 2 brothers and 2 sisters all had positive D1853N polymorphism in ATM gene as a predisposing factor. Positive or negative status of this polymorphism will be,respectively either unlucky or lucky, exactly similar to be, or not to be which initially stratifies the screening management, not only for the proband as the first target member, but for all relatives, and the net generation through the target pedigree.This is an influential and translatable bridging system between the past, present and future destiny within the pedigree.

Peripheral blood cells of a patients affected with with VHL, at early stage of disease is provided in Figure **2**, in which the arrows refer to the migrated tumor cells into the blood stream of the VHL-patient.

By focusing on actual initial management, prompt screening at embryonic and fetal levels have beneficial impact through the pregnancy; by applying the periodic and prenatal diagnosis for any further essential clinical strategies through circulating embryonic and/or fetal cells during the pregnancy. Such platform is a rapid,
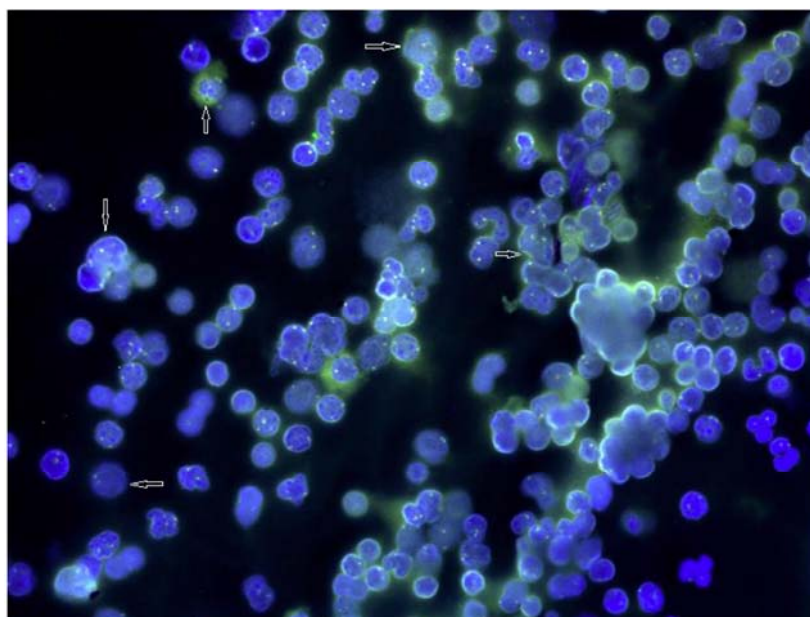

Figure 2: Peripheral blood cells of a patients affected with with Von Hippel lindau, at early stage of disease. Magnification: x100, arrows refer to the circulated tumor cells into the blood stream.

and non-invasive screening to apply: 1) further complementary molecular investigation; 2) the functional alterations of the involved protein (s), and 3) the prompt personalized and target- based clinical management (s). As a matter of fact, the current cancer management is always late, because of the hidden evolutionary course all through the cancer initiation, development, and progression from zygotic, through early embryonic period, all through the fetal duration, and post-natal (PN) periods.

Furthermore to highlight the key role of an early detection, tracing the migrated neural cells to the blood stream of patient affected with VHL is provided (Figure **3**). The PN is the complementary period, with many hidden hazards, which severely restricts the health status of the borne case. The micro- and macro-environmental factors, hands in hands, help to progress the cancer evolution which has been initiated at zygotic and/or embryonic/ Fetal (ZEF) periods, and progresses through the post-natal life. Therefore, the entire environmental factors, including maternal-nutrition, milk, and beyond are the essential to be considerations and controlled. Another focal and influential factor is the psychological status, all through the parental side and beyond which affects the psychological mode of fetus, at pre-, during- and post-natal periods.

In spite of diverse degree of expression between three different cell cycle's targets, remarkable co-expression between PCNA, cyclin E and cyclin B reflect a significant cooperation to facilitate cell cycling progression. Figure **4** presents the end stage production of cell cycle, i.e., protein expression in the oocytes is an extremely early detection and a remarkable predictive and preventive approach in the maternal circulating sample, with aim of further follow up exploration all through the pregnancy. Such an early prenatal platforms will be the guide for further plan and consideration for minding the micro-, and macro-environmental factors, during pregnancy and milking period, and all through the life periods of mother and fetus.

The sampling is performed at an earlier period of being diagnosed as affected with Von Hippel lindau in which few circulating neural cells could be detected (marked with arrow). the pedigree analysis of the referral case provided the following information:

The proband is affected with VHL. His Father, mother, 2 brothers and 2 sisters all have positive D1853N polymorphism in ATM gene, as a predisposing factor which play a prognostic and predictive role in this family.

The neural cells are characterized with 2 signals as G2 (marked with star), and cells containing both signals including G1 and G2 (are referred with arrows). A CNC (a remarkable circulated neural cells, marked with a circle) is at Synthesis phase. The cells with both G1 and G2 are characteristic as multi- phase.
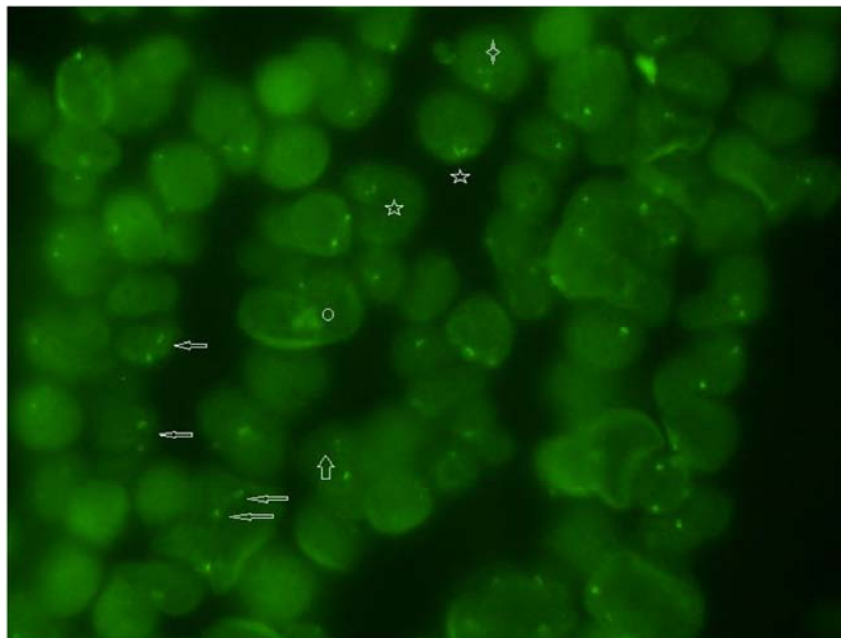

Figure 3: Circulating neural cells from a patient affected with Von Hippel Lindau syndrome (VHL) in the blood stream territory M blood cells are in the Growth 1 or growth 2 of cell cycle (magnification:x1000).
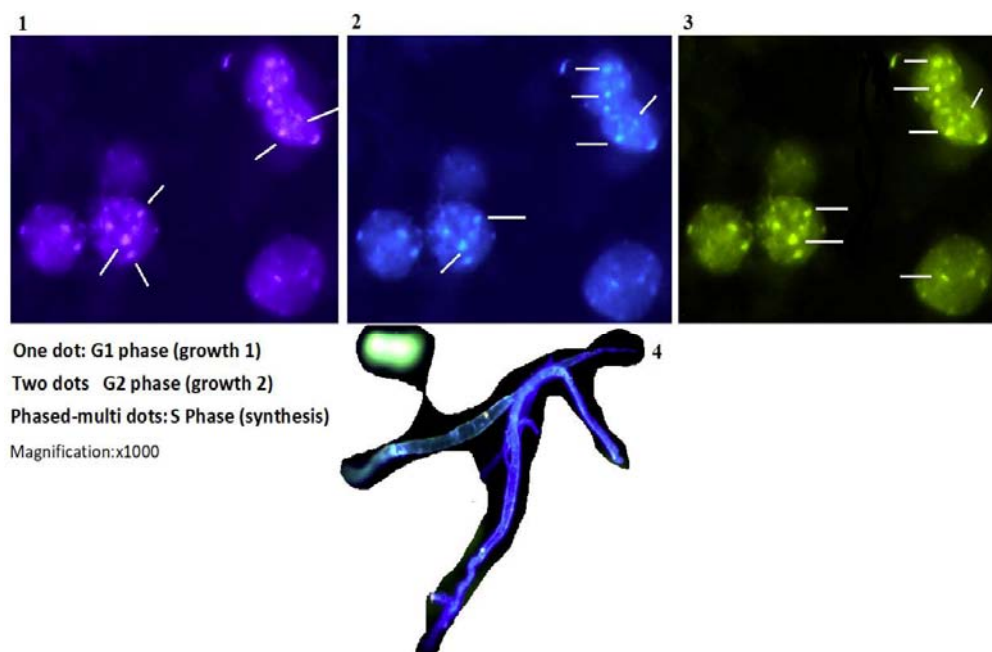
Figure 4: Circulating brain tumor cells in the peripheral blood sample of a patient affected with brain tumor.

Image 1: neural cell conjugated with neural specific antibody, dapi and cy5; image 2 is conjugated with dapi/ FITC, and image 3 is conjugated with dapi/CCND1/FITC; and image 4 presents the circulating tumor cells, conjugated with Dapi/ CCND1/Neural cell marker.

Combination of different phases are available bellow the figure. Including G1 phase as a solitary dot, G2 is defined as two dots, and mutti -dots present S phase.

Cells are conjugated with FITC, as an antibody for neural growth factor in neural cells.

Regarding brain tumor, multi-phase evolutionary based characteristics has been also unmasked in different BTs, at single cell level. Image of the circulating brain tumor cells in the peripheral blood sample of a patient affected with BT is also provided (Figure **4**) in which the combination of different phases are available.

By focusing on the early detection, the accessible destinations include: 1) available oocytes (Figure **4**), 2) embryonic, 3) fetal and 4) chorionic samples are considered as: the quadrant directive, predictive and early diagnostic platforms which could be non-invasively explored, as the valuable target based strategy all through the periods of pre- stage and through the pregnancy.

Expression and co-expression of proliferative nuclear antigen, cyclin E and cyclin B in oocytes of the embryogenic process are provided (Figure **5**).

This image presents expression of **a**) proliferative nuclear antigen (PCNA), **b**) cyclin E; **c**) cyclin B and **d**) co-expression - of PCNA/cyclin E/cyclin B

Images a and b include only 2 cells with high expression, including PCNA and cyclin E. Image c have more cells with higher expression; and d) present remarkable co-expression between PCNA, cyclin E and cyclin B. Bar:20 µm.

Furthermore, Expression of proteins CD44, CD24 and vascular endothelial growth factor (VEGF) have been explored in the spontaneously discharged sample of an embryo which provides the new insight for further predictive strategy. As Figure **6** presents, there is more harmonic expression between CD44 and VEGF than CD44 with CD24. In addition, there are limited positive stem cell, i.e., CD44+/CD24- which has been marked in images b and d (Figure **6**).

Expression of proteins CD44, CD24 and VEGF have been explored in the spontaneously discharged sample. As Figure **6** presents, there is more harmonic expression between CD44 and VEGF than CD44 with CD24. In addition, there are no positive stem cell, i.e., CD44+/CD24-(Figure **8**).

Lack of expression of CD133 reveals that the formation of neural stem cells has not occurred at the early stage of embryonic period . However, the VEGF as an essential target for cellular growth has been activated at 5th weeks of gestation (Figure **7c**). This
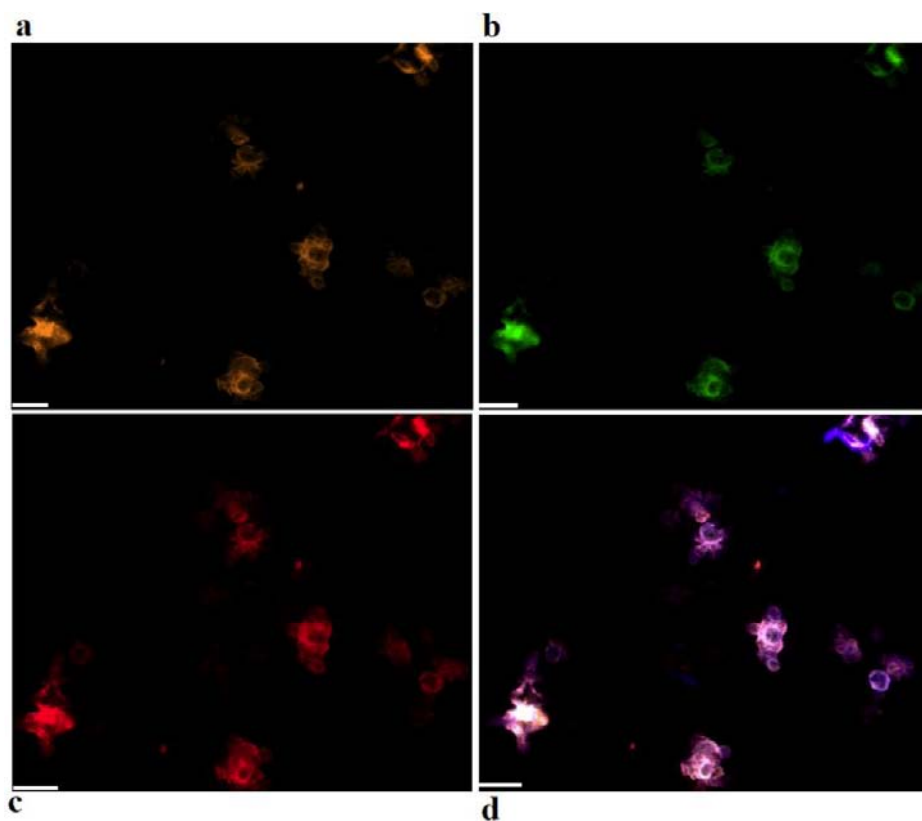
Figure 5: Expression and co-expression of proliferative nuclear antigen, cyclin E and cyclin B in oocytes of the embryogenic process.

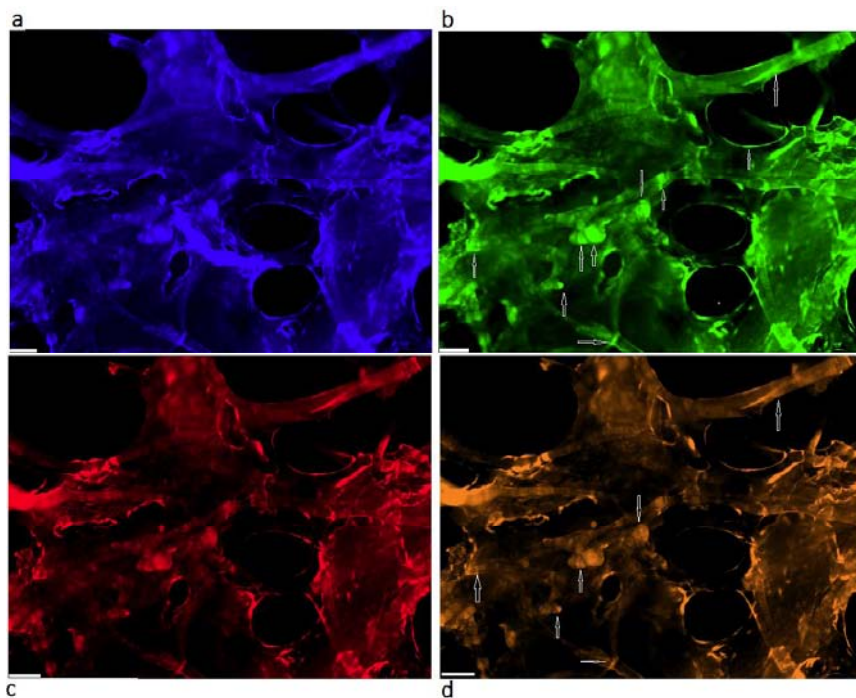a: PCNA; b: Cyclin E; c: cyclin B, d:co-expression of PCNA/E/B.

Figure 6: Expression of CD44, CD24 and VEGF in embryonic cells.

Stem cell of CD44, CD24 and in the middle stage of embryonic discharged sample.

a. Embryonic (Em) cells with dapi; b. Em cells conjugated with CD44/FITC; c: Em cells conjugated with VEGF; and d. Em cells conjugated with CD24/Rpe.

VEGF: vascular endothelial growth factor. The cells characterized as CD44+/CD24- are considered as the positive stem cells. Bar:20 μm.
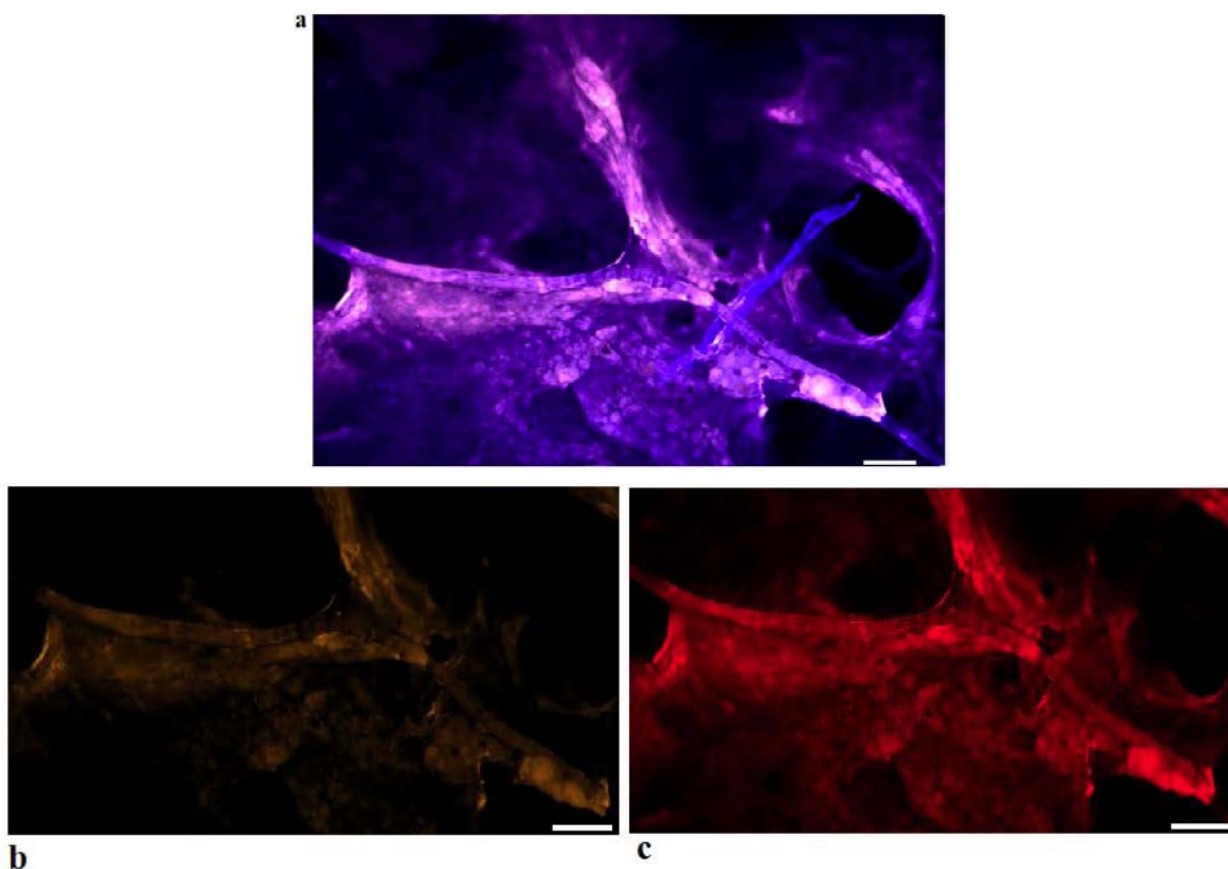
Figure 7: Expression of the neural antibody, CD133 and vascular endothelial growth factor.

a. Embryonic (Em) cells with Dapi/CD133/VEGF; b. CD133, as neural stem cells (negative status), and c. same cells conjugated with VEGF/Pe-cy5:vascular endothelial growth factor. Bar:20 μm.

finding has beenn, initially traced in in the circulating neural cells as well.

Diagnosis of CD44+/CD24- is considered as a positive stem cell, (Figure **6**). This reliable diagnosis may be a valuable chance in clinical aspects in both fields of oncology and psychology for the target patients. In addition, it may provide a supportive impact to be considered as a routine test in clinical oncology. Furthermore, the similar impact is applicable for CD133 for being positive, or negative, or mosaic in Neurological based neoplasms including brain tumors (Figure **7**) and Von Hippel Lindau syndrome. Lack of expression in stem cell CD133 at 5th weeks of embryonic level is traced which is an applicable finding in cancer management.

Status of CD44/CD24 has been assayed in the discharged embryonic sample which reflects a mosaic status (CD44+/CD24- as positive and mosaic pattern including CD44+/CD24- as positive-accompanied by CD44+/CD24+ as negative) (Figure **6**) The Stem cells, such as CD44/CD24 are the reliable and predictive factors.

Cell Cycle Harbors a Translatable Multi-Phase Based Reservoir

Hypothesis of Multi-Phase (MP) Phenomena in breast cancer as an evolutionary and natural art- based process through the cell cycle has already been delivered (Mehdipour, 2022).

Interestingly, MP event has also been traced in different tissues including buccal cells, chorionic villus sample (CVS), and all tissues of the body including brain, alimentary system, etc.

As a sample case, the image in CVS is presented (Figure **8**). The arrows in the middle of figure and at bellow/righ refer to the presence ofG1 and G2 signals.

The Basic Events through the Cell Cycle at a Glance

- Phosphorylation of pRb in G1 is performed through cyclin D/CDK-4 or 6, and is completed at early G1.

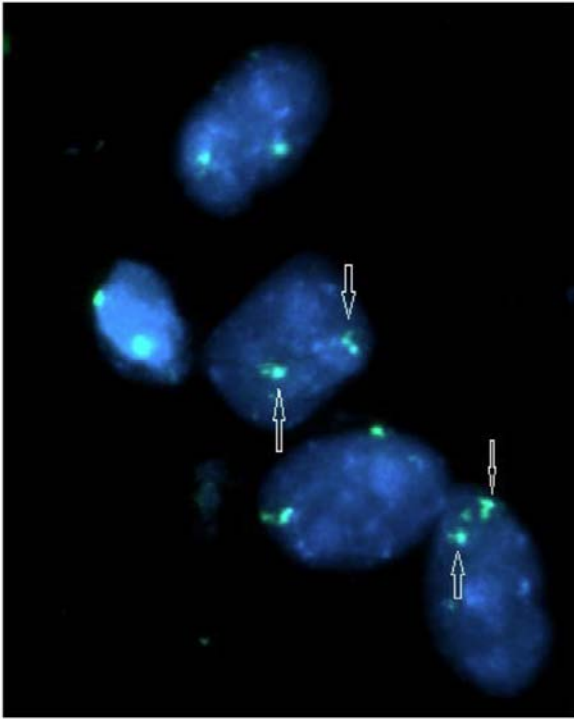- Activation of CDK2,by cyclin E is aimed to phosphorylate pRb in late G1.

Figure 8: Dual phase in chorionic villus cells.

Single and dual phases include G1 and G 2 respectively, accompanied by single phase, as G1 (top/left).

Magnification:x1000.

- Complex formation of cyclin A/CDK in S phase.

- The Cyclin B1/CDK1 complex leads to transitional process through G2-M phases.

- Heterogeneity and course of evolution are, basically, unveiled in metaphase or in interphase by relying on the traditional data sets including both classic cytogenetic and molecular based data.

The functional data is characterized : 1) as the final mechanisms, and core data through the cell cycle territory; 2) to offer the novel characteristics with applicability in early/personalized- Diagnosis/ Prediction/Prognosis/Prevention/therapeutic, as the– formulated- periodic chart.

In addition, the novel evolutionary based platform as the mosaic, combined phases at single cancer cells have offered new platform for the personalized diagnosis and clinical managements including therapy [1] at single cell level. This platform as the "Complex/*Mosaic Phases* (*CMP*)" has offered the personalized translatable insight through an original and individuaalized cell cycle based therapy. In fact, cell cycle is a powerful centralized destination to contour the initiation, development and therapeutic tactics of the neoplasia, but this process requires the parallel cellular destinations including. Telomere, telomerase, Sub-telomere, Epigenetics, and chromosomes [13-16].

The MPs are characterized as:

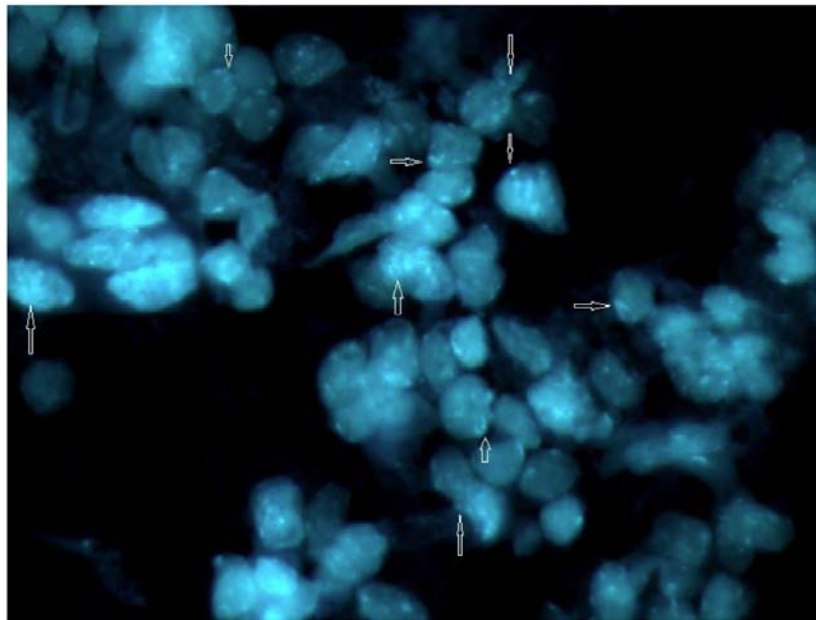- Micro Chimers, as the mirror of the hidden and sequential manner of cancer cells.

Figure 9: An un-differentiated Brain tumor cells with divers multi-phase characteristics.

Mosaicism is characterized with diverse frequency including single, double and multi- signals.

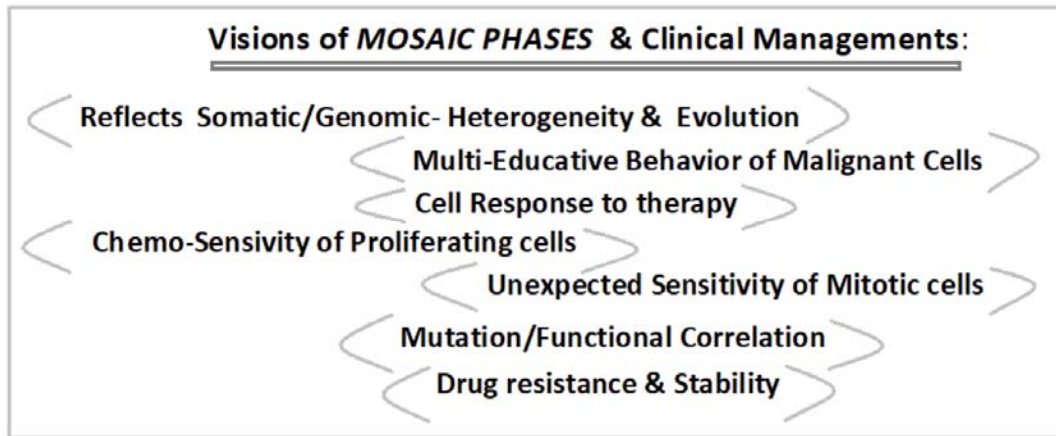Tumor cells are characterized with cells containing diverse signals,Magnification:x1000.

Figure 10: Mosaic phases and clinical managements.

- Similar as the stem cells such as co-expression of CD44+/CD24- versus 4G1/1S (Figure **6d**).

Finally, this podium, initially offers the single cell based identification at cell cycle level, aiming an individualized strategy, flavored with mathematics-based therapeutic mode.

CONCLUSION

By an initial publication of the novel *Mosaic Phases (MPs)* hypothesis in the primary breast cancer patients it was aimed to provide a broader spectrum of this hidden event in the diverse neoplastic disorders including cancer. That is an evolutionary based phenomenal behavior as, forward and backward.

MPs are specialized to mask their territorial secrets as a historical souvenir which has been unmasked as the highlight of the evolutionary course.

MPs definitely offer the beneficial podium for the neoplastic disorders.

Enlightening the cell cycle territory lead to an educational platform, with aim of revolutionizing the alphabetic order of cell cycle which will revolutionize the cancer management including Personalized, Predisposing, Prediction, Prognosis, Prevention, as 5xP, an extremely early detection, and early therapy.

MPs are capable to match the specific CDKs to the matched/target cyclins by considering the high cellular enumeration. Upon such regulative strategy, the initial core decision for therapeutic platform would rely on the cell cycle- MPs guide, according to the backward/forward route in the broad spectrum within the neoplastic classification. Finally, the highlight of an early cancer management from diagnosis to therapy may rely on MPs cell cycle model, accompanied by any additional abnormal molecular- and functional based of

Figure 11: Graphic abstract.

the related markers. Furthermore, the pedigree based analysis is an essential step to ease and categorize any history of the genetic-based disease(s) through different generations; and considering micro- and macro-environmental factors, including hazard systems and nutrition, from embryo to post- natal periods. Finally, application of the cellular and molecular abnormities (inherited, and /or acquired) for an early detection and screening purposes is required.

At a glance, the Multi-Phase based signature provides a reliable platform for the personalized cancer management. The focal targets included 1) the neural cells in three diverse neoplastic complications, including Brain tumor, Leukemia, and Von Hippel Lindau syndrome. The roots including embryo and chorionic villus samples have been explored by non-invasive sampling including by embryonic and chorionic villus samples. Finally, the most reliable diagnostic platform, that is in 2ml of maternal circulating blood sampling were applied for the prenatal diagnosis of circulating embryonic cells (CEmCs)and Chorionic villus cells(CVCs) (Figure **11**).

COMPETING INTERESTS

The author disclose any competing interests that are directly or indirectly related to the present work submitted for possible publication.

REFERENCES

[1] Mehdipour P. Evolutionary Hypothesis in Cell Cycle of Breast Cancer Patients: Mosaic Phases in Single Cancer Cells. Journal of Cancer Research Updates 2022; 11: 43-53. https://doi.org/10.30683/1929-2279.2022.11.07

[2] Mehdipour P. Predisposing, preventive, predictive cancer marker kit (3xPCM) (International Application No.: PCT/IB2014/065072) 2006. Patent ID:WO 2016/05529A1

[3] Belanger H, Beaulieu P, Moreau C, Labuda D, Hudson TJ, Sinnett D. Functional Promoter SNPs in Cell Cycle Checkpoint Genes. Human Molecular Genetics 2005; 14: 2641- 8. https://doi.org/10.1093/hmg/ddi298

[4] Dickson MA. Molecular pathways: CDK4 inhibitors for cancer therapy. Clinical Cancer Research 2014; 20: 3379-3383. https://doi.org/10.1158/1078-0432.CCR-13-1551

[5] Dalton S.Linking the cell cycle to cell fate decisions. Trends in Cell Biology 2015; 25: 592-600. https://doi.org/10.1016/j.tcb.2015.07.007

[6] Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, Seal S, Ghoussaini M, Hines S, Healey CS, Hughes D. Genome-wide association study identifies five new BC susceptibility loci. Nature Genetics 2010; 42: 504-507. https://doi.org/10.1038/ng.586

[7] Siegel PM, Hardy WR, Muller WJ. Mammary gland neoplasia: insights from transgenic mouse models. Bioessays 2000; 22: 554-563. https://doi.org/10.1002/(SICI)1521-1878(200006)22:6<554::AID-BIES8>3.0.CO;2-A

[8] Salmon ES, Sartorelli AC. Cancer Chemotherapy. In: Katzung. BG, editor. Basic & Clinical Phamacology, Appleton & Lange. UK 1998; 881-915.

[9] Santamaria D, Barriere, C, Cerqueira, A, *et al.* Cdk1 is sufficient to drive the mammalian cell cycle. Nature 2007; 448: 811-815. https://doi.org/10.1038/nature06046

[10] Schwanitz G, Raff R. Application of specific cytologic, cytogenetic and molecular-cytogenetic techniques for the characterization of solid tumors. Annales Academiae Medicae Bialostocensis 2005; 50: 91-96.

[11] Ghavamzadeh A, Alimoghaddam K, Mehdipour P, Sharifian R, Schwanitz G, Shamshiri AR. Transformation of chronic myelogeneous leukemia to multiple myeloma: a case report. Acta Medica Iranica 2003; 41(1): 45-49.

[12] Cresspigio J, Berbel LCL, Dias MA, Berbel RF, Pereira SS, Pignatelli D, Mazzuco TL. Von hippel lindau disease. A single gene, several hereditary tumors. J Endocrinol Inves 2018; 41(1): 21-31. https://doi.org/10.1007/s40618-017-0683-1

[13] Kang JH, Kim SJ, Noh DY, Park IA, Choe KJ, Yoo OJ, *et al.* Methylation in the p53 promoter is a supplementary route to breast carcinogenesis: correlation between CpG methylation in the p53 promoter and the mutation of the p53 gene in the progression from ductal carcinoma in situ to invasive ductal carcinoma. Lab Invest 2001; 81: 573-9. https://doi.org/10.1038/labinvest.3780266

[14] Gonzalez-Gomez PB, MJ. Lomas J. Arjona D. Alonso ME. Amiñoso C. Lopez-Marin I. Anselmo N.P. Sarasa J.L. Gutierrez, M. Casartelli, C. Rey JA. Aberrant methylation of multiple genes in neuroblastic tumours; and relationship with MYCN amplification and allelic status at 1p. Eur J Cancer 2003; 39: 1478-85. https://doi.org/10.1016/S0959-8049(03)00312-5

[15] Mehdipour P, Karami F, Javan F, Mehrazin M. Linking ATM promoter methylation to cell cycle PE in brain tumor patients: cellular molecular triangle correlation in ATM territory. Molecular Neurobiology 2015; 52: 293-302. https://doi.org/10.1007/s12035-014-8864-9

[16] Knudson AG. Mutation and cancer: statistical study of retinoblastoma.Proceedings of the National Academy of Sciences 1971; 68: 820-823. https://doi.org/10.1073/pnas.68.4.820