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The Complexity of Tumor Heterogeneity: Limitations and Challenges of the Pharmacogenomics in Cancer Treatment

Guglielmo Militello¹, Marta Bertolaso²

Abstract

One of the most important current applications of personalized medicine is the study of the correlations between genetic variations and the emergence of tumor phenotypes in specific human groups in order to provide more personalized treatments. In particular, pharmacogenomics aims at studying the relationship between drug response and individual genomic variations. In cancer treatment, however, the overall effectiveness of this gene-based approach is impaired by the genetic and phenotypic heterogeneity of tumor cells, thus making the pharmacogenomics in cancer treatment problematic. The purpose of this paper is threefold: first, to analyze the levels of organization of tumor heterogeneity; secondly, to explore the complexity of tumor heterogeneity; finally, to estimate the epistemological and practical consequences of such a complexity for the pharmacogenomics in the personalized cancer treatment.

Keywords: Intratumor Heterogeneity; Cancer Complexity; Pharmacogenomics; Personalized Medicine; Systems Pharmacology

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1. Introduction

Over the last two decades, pharmacogenomics has been attracting sizeable investments from both the public and the private sector. The primary purpose of pharmacogenomics is to identify the variant genes affecting drug response and to develop drugs that are based on the genetic profile of a patient. As such, pharmacogenomics is currently considered a fundamental lynchpin of personalized medicine, which is an application of molecular and/or genomic data in medicine in service of diagnosis, prognosis, or treatment. Pharmacogenomics has already been employed to study the precise dosing of drugs for the treatment of different pathologies (e.g., cardiovascular diseases, osteoarticular pathologies, psychiatric disorders) (Ranganathan 2008; Joyner and Paneth 2019).

A promising field of application of pharmacogenomics is the treatment of cancer, as carcinogenesis is thought to be caused or, at least, closely connected with genetic mutations. Thus, pharmacogenomics aims at discovering the molecular targets of anti-cancer drugs, in order to make them more effective. Nevertheless, tumor cells exhibit genetic and phenotypic *heterogeneity* that seriously interfere with the molecular targets of anti-cancer drugs. Indeed, genetic heterogeneity could enable tumor cells to bypass or halt the drug's progress, and phenotypic heterogeneity can make getting drug to target difficult (e.g., it can prevent the drugs from diffusing into a tumor).

It therefore seems that a successful application of pharmacogenomics to cancer treatment hinges on the knowledge of its levels of organization. Accordingly, the purpose of this paper is threefold: first, to analyze the levels of organization of tumor heterogeneity; second, to examine the complexity of intratumor heterogeneity; finally, to evaluate the consequences of such a complexity for the epistemology and scientific practice of pharmacogenomics in the context of personalized cancer medicine.

The philosophical literature usually considers “complexity” as a feature of *systems* (e.g., physical, biological, social systems) (Ladyman et al. 2013). However, since tumor heterogeneity is not a system, but rather a property (or *phenomenon*) of a system such as cancer, is it appropriate to define tumor heterogeneity as “complex”? In our view, the answer is *positive*, because (as we will argue in section 4) tumor heterogeneity is a very complicated phenomenon both ontologically and epistemologically: it occurs at different organizational levels and involve heterogeneous entities that are causally interdependent, thus establishing an intricate network of causes and effects. As a consequence of such an ontological complexity, there is an *epistemological* complexity that mostly entails the difficulty in explaining the intricate network of causal relationships underlying genetic and phenotypic heterogeneity.

The understanding of the complexity of tumor heterogeneity will allow us to critically assess the limitations, the challenges, and the potentialities of the theoretical framework and research

programs of pharmacogenomics in cancer treatment. We argue that tumor heterogeneity can adequately be addressed by considering the (causal) relationships between different levels of organization of cancer and it could also be stopped or, at least, slowed down by exploiting systemic properties of cancer. We suggest that a gene-based approach to anti-cancer drugs is neither the most effective nor the most relevant way to deal with tumor heterogeneity and cancer progression, and that pharmacogenomics may benefit from the tools of systems pharmacology.

The paper is organized as follows. Section 2 conducts a critical review of the main theoretical accounts of pharmacogenomics, focusing on its promises and its main obstacles. In the Section 3, we examine the levels of organization of tumor heterogeneity. Section 4 explores the complexity of tumor heterogeneity. Then, Section 5 evaluates how the complexity of intratumor heterogeneity affects the epistemological framework and the scientific practice of pharmacogenomics for cancer treatment. Finally, section 6 makes some concluding remarks.

2. The Promises and the Obstacles of Pharmacogenomics: A Critical Review

From an historical point of view, the rise of personalized medicine is directly linked to the Human Genome Project³, the Human Haplotype Map Project⁴, and the development of -omics data such as genomics, transcriptomics, proteomics and metabolomics (Ghosh and Poisson 2009). Personalized medicine aims at integrating the data coming from genomics with the clinical data of individual patients in such a way as to create very specific therapies (and drugs) based on the genetic profile of each patient (Emmert-Streib 2013). As such, personalized medicine is usually regarded as an evidence-based medicine, because it relies on an evidence hierarchy (e.g., cohort studies, randomized control trials, meta-analyses), and also as a precision medicine, since it encourages a model of healthcare grounded on medical decisions, treatments, and drugs that are individually tailored (Joyner and Paneth 2019).

An essential aspect of personalized medicine is *pharmacogenomics*⁵, which aims at developing drugs on the basis of the genetic profile of a patient. The main *theoretical assumption* underlying pharmacogenomics is the idea that a drug is likely to be more efficacious and less toxic if the genetic profile of a patient is fully known and if the genetic variability associated with a certain

³ The Human Genome Project was an international research project, carried out between the 1990s and the early 2000s, that aimed at sequencing all the genes of the human genome.

⁴ The Human Haplotype Map Project was an international project carried out between 2002 and 2009 that aimed at developing a haplotype map of the human genome so as to explain the main genetic variants causing diseases and responses to drugs (International HapMap consortium et al. 2010).

⁵ The first steps towards pharmacogenomics can be traced back to the 1950s, as a number of scientists tried to merge genetics, biochemistry and pharmacology into a new scientific discipline (Mancinelli et al. 2000). Afterwards, the human genome project played a fundamental role in opening up a whole new field of research in pharmacogenomics (Ginsburg and Willard 2009).

disease and some drug response is perfectly understood (Karczewski et al. 2012). As such, drug therapy in pharmacogenomics entails a division of patients into smaller subpopulations each having specific genetic profiles. Pharmacogenomics has a twofold aim: firstly, to identify the *variant mutations* affecting the response to drugs in individual patients; secondly, to develop *new drugs* based on the discovery of new drug targets (Mancinelli et al. 2000).

The identification of genetic mutations combines different techniques that are aimed at studying not only the gene targets for some drugs but also the regulatory and signaling networks in which the gene is embedded. One of the most important methods is genome-wide associations studies (GWAS) that examine the correlation between single nucleotide polymorphisms (SNPs)⁶ and phenotypic traits involved in human diseases (Lu et al. 2014)⁷. Other techniques include expression methods, cheminformatics, and pathway discovery (Karczewski et al. 2012). Expression methods detect the genes involved in drug response by means of RNA expression data from microarrays or RNA-Seq⁸ technique from drug-treated samples (Karczewski et al. 2012). Different expression profiles are then compared through the connectivity map, which shows the gene expression data of cell lines in terms of small molecules. A number of computational methods (i.e. cheminformatics) combine information about protein structure and small molecule structure for understanding potential drug targets.

Pharmacogenomics plays a fundamental role in the development of new drugs, as it can help to identify new potential gene targets of drugs. Through the use of next generation sequencing (a set of techniques aimed at sequencing the whole genome) in a certain cohort of patients, the current drug development efforts seek to pinpoint the genetic variants responsible for therapeutic vulnerability, to synthesize molecules that can inhibit the activated protein variants, and to identify additional patients who could eventually benefit from these targeted therapies (Mardis 2019).

Pharmacogenomics has brought some considerable benefits for the treatment of some pathologies (e.g., cardiovascular diseases, osteoarticular diseases), because the clinical trials of pharmacogenomics have made it possible a more precise dosing of some drugs (Ranganathan 2008; Joyner and Paneth 2019). However, pharmacogenomics has important *limitations* due to the “dynamic complexity of the human genome, multigenic disease origins, and involvement of numerous genes in drug response” (Mancinelli et al. 2000, p. 11). Indeed, most noninfectious

⁶ Single nucleotide polymorphisms are substitutions of single nucleotides in a certain DNA strain.

⁷ GWAS usually employ control setups and the analysis of quantitative phenotype data. The former is an observational study that compares a set of healthy individuals with another set of individuals affected by a disease by analyzing the *alteration of allele frequency* in the SNPs of the ill patients. The latter is the quantitative analysis of biomarkers concentrations or gene expression in order to evaluate the amount of mutated phenotypes in the cluster of ill people (Bush and Moore 2012).

⁸ RNA-seq stands for RNA sequencing which employs the technique of next generation sequencing for analyzing the presence and the concentration of RNA in a sample.

diseases (e.g., cardiovascular, renal diseases, etc.) are the outcome of many gene variants working collectively in such a way that it is impossible to find a unique genetic cause (Kalow 2006). Accordingly, some doubts have arisen over the effectiveness of the personalized medicine and somebody has voiced a fear that “the concentration of research on personalized medicine might deprive other promising avenues of research of appropriate resources” (Tannock and Hickman 2016).

In the case of cancer cure, the application of pharmacogenomics to the treatment of malignant tumors reveals not only some potentialities, but also some important limitations. On the one hand, pharmacogenomics is a promise for the treatment of cancer, insofar as the creation of more effective anti-cancer drugs is believed to depend on the mechanistic understanding of their gene targets. A number of different parameters (e.g., SNPs, copy number variations (CNV), gene deletion and insertion) are evaluated to assess how anti-cancer drugs are metabolized in different subpopulations of patients (Dhawan and Padh 2013). On the other hand, it has recently been shown, for example, that the percentage of US cancer patients that benefitted from pharmacogenomics is very low (Marquart et al. 2018).

A fundamental aspect of cancer cells that hinders pharmacogenomics lies in that the cells of the *same* tumor are extremely heterogeneous (intratumor heterogeneity)⁹. A fundamental aspect of cancer cells that hinders pharmacogenomics lies in that the cells of the same tumor are extremely heterogeneous (intratumor heterogeneity). Basically, intratumor heterogeneity refers to the differences in gene expression (e.g., the expression of cell surface markers or growth factors), morphology (e.g., solid structures, tubular structures, alveolar structures), and other cellular capacities (e.g., metabolism, motility, immunological capacities) between the cells of the same tumor (Marusyk and Polyak 2010) often including mutations related to cell-cell communication and differentiation. A second sense of tumor heterogeneity is related to the genetic drift¹⁰ of tumor cells, which “refers to the equally important source of cellular variability in cancer derived from genetic heterogeneity in a tumour that accumulates as cancer progresses. [...] The processes of increasing tumour heterogeneity imply the disruption of the correct processes of differentiation in the progenitor tumour cell with a concomitant loss of control on the genomic level of organization in tumour cells” (reviewed in Bertolaso 2016, p. 14). The heterogeneity that is generated in this way usually follows the previous one (i.e. functional heterogeneity) posing interesting questions about

⁹ Another important dimension of the heterogeneity of cancer is the genetic and morphological differences between the cells of different tumors (inter-tumor heterogeneity). In spite of its importance, we focus in this paper on intratumor heterogeneity and on its epistemological and practical challenges to pharmacogenomics.

¹⁰ Genetic drift is the change in the frequency of gene variants (alleles) in a population.

the fundamental organizational features that are actually compromised in cancer and their reciprocal temporal dependencies in causal terms.

A major consequence of intratumor heterogeneity is that tumor cells exhibit new adaptive behaviors by interacting in a new way with their microenvironment. When they are targeted by anticancer drugs, they develop resistance to single molecular targeted genes by means, for example, of epigenetic upregulation¹¹ of partially inhibited pathways, mutation of the gene target, or activation of alternative pathways (Tannock and Hickman 2016). Adaptive responses often involve multiple targets, and also it can be very difficult to combine targeted agents (Tannock and Hickman 2016). One of the most dramatic effects of tumor heterogeneity is that cancer cells, when treated with anti-cancer drugs, produce bottlenecks in which some drug-resistant cells survive, thus proliferating and generating metastasis (Marusyk and Polyak 2010). Anticancer treatments provide only a partial inhibition of some signaling pathways involved in tumor proliferation, and also they are extremely toxic and cannot be used in combination (Tannock and Hickman 2016).

In view of the foregoing, the essential question for the application of pharmacogenomics to cancer treatment is whether it is possible to develop efficacious gene-based drugs in spite of intratumor heterogeneity (Tannock and Hickman 2016, p. 1292). For gene-based drugs to be successful, they should be able, firstly, to target the mutations driving tumor genomic instability and tumor heterogeneity, and secondly to inhibit the pathways of tumor progression (Tannock and Hickman 2016). We may speculate that the biological properties and the specific organization of tumor heterogeneity pose big challenges to the current epistemic paradigms and the overall scientific practice underlying pharmacogenomics. Thus, in order to better evaluate the obstacles and potentialities of pharmacogenomics in cancer treatment, we carefully analyze in the next section the levels of organization of tumor heterogeneity as they are represented in the scientific literature.

3. Levels of Organization of Intratumor Heterogeneity

It was not until the 1930s that the existence of morphological and functional heterogeneity of cancer cells was revealed, inasmuch as it was shown that the transplantation of one leukemia cell of mouse was able to originate new malignant leukocytes with new morphological and functional traits (Furth and Kahn 1937). During the 1950-60s, cytogenetic studies on the allelic assessment of metabolic isoenzymes¹² and immunoglobulins¹³ as well as on DNA polymorphisms confirmed the idea that one single mutated cell gave rise to new malignant cells with different genetic and phenotypic

¹¹ Epigenetic regulation plays a pivotal role in the transcriptional regulation of cancer. More particularly, epigenetic upregulation of DNA repair genes entails the increase in gene expression, whereas epigenetic downregulation a decrease.

¹² Isoenzymes (or isozymes) are enzymes with different amino acid sequences but catalyze the same chemical reaction.

¹³ Immunoglobulins (or antibodies) are the proteins synthesized by the plasma cells of the immune system.

profiles (Loponte et al. 2019). Since the 1970s, the genetic and phenotypic heterogeneity of cancer cells has been investigated within the theoretical framework of the “Somatic Mutation Theory” (SMT) which posits that carcinogenesis and the proliferation of cancer cells is caused by somatic mutations (i.e. alteration of the DNA sequence of the cells of a multicellular organism). At the end of the Nineties, an alternative explanation of tumor heterogeneity has been provided by the “Tissue Organization Field Theory” (TOFT) which states that the initiation and proliferation of cancer is determined by some tissue dysfunctionalities that affect the genetic organization of cells. Furthermore, systemic accounts of tumor heterogeneity have recently been provided by scholars studying cancer dynamics with the tools of Systems Biology. In both accounts, intratumor heterogeneity has inextricably been linked to the factors responsible for *carcinogenesis* and *tumor progression*; yet, their different explanations of the causes of cancer initiation and progression inevitably affect how they describe genetic and phenotypic heterogeneity. Thus, the next two subsections examine intratumor heterogeneity in the context of the SMT, firstly, and then in the TOFT and in the systemic models in cancer research.

3.1 Intratumor Heterogeneity from the Perspective of the SMT

Although the SMT has been formulated in distinct ways¹⁴, some biological assumptions form its innermost core. Firstly, neoplasms are characterized by *genetic mutations* that increase their proliferation rate. Secondly, the *cell* is regarded as the fundamental level to explain carcinogenesis and tumor progression. Thirdly, tumors stem from a single cell that has acquired multiple mutations and has the potential for unlimited proliferation. Finally, neoplasms arise when there are genetic mutations or increased expression in those genes (the *oncogenes*) regulating cell growth and differentiation (reviewed in Bertolaso 2016, p. 20). According to the SMT, *genetic* and *epigenetic* factors are the primary causes of intratumor heterogeneity, which in turn favor tumor evolution¹⁵ and tumor progression¹⁶.

The genetic factors contributing to intratumor heterogeneity include changes in both the single genes (e.g., nucleotide polymorphisms, heterozygosity, splicing forms, etc.) and in the whole genome (e.g., copy number variation, microdeletions and inversions, aneuploidy, polyploidy, etc.) (Heng et al. 2009; McGranahan and Swanton 2017). Some studies have underlined that tumor

¹⁴ For a detailed discussion of the different accounts of the SMT, see Bertolaso (2016, pp. 17-41).

¹⁵ By *tumor evolution*, we mean the dynamics of natural selection that underlie the progression of neoplasms.

¹⁶ The expression *tumor progression* is employed in this paper to refer to the *growth* and *proliferation* of tumor cells. We will not use the term *development*, because this word usually relates to a gene-controlled process of self-organization both in time and space, whereas cancer progression is driven by stochastic events of gene replacement which do not follow regulated patterns. In spite of being correlative terms, tumor *progression* and tumor *evolution* designate different biological aspects of cancer dynamics.

heterogeneity is likely due not only to random genetic mutations, but also to the recurrent inactivation of the same tumor suppressor genes (Gerlinger et al. 2012; Swanton 2012). Furthermore, some genetic events (e.g., polyploidy, chromosomal instability) could explain the initial phases of tumor growth and intratumor heterogeneity, while other mutations (e.g., the mutations of p53¹⁷) could drive tumor growth and maintenance (Swanton 2012). Altogether, these genetic mutations effect a profound transformation in how a healthy cell synthesizes proteins, performs (genetic) regulatory mechanisms, and generates intra- and extra-cellular signals.

A huge variety of epigenetic mechanisms yield intratumor heterogeneity, the most important of which are changes in DNA methylation, reorganization of chromatin, variability in gene expression and microRNAs¹⁸ (Heng et al. 2009; Gerashchenko et al. 2013; Mazor 2016). DNA methylation increases the genomic instability as indicated by the aberrant methylation of the promoter genes of CpG island¹⁹ methylator phenotype that is involved in cell control and development (Gerashchenko et al. 2013, p. 1203). The remodeling of chromatin is due to post-translational modifications of histone proteins and modifies gene transcription. This has some important cellular effects, inasmuch as, for instance, the deacetylation of histones can negatively affect cell adhesion and intercellular contacts, thus generating different morphological structures within the tumor (Gerashchenko et al. 2013, p. 1203). MicroRNAs play an important role for the diversity of tumor cells, because variations in microRNAs determine an alteration in the regulation of cell cycle and differentiation of healthy cells, thus promoting cancer differentiation and progression (Gerashchenko et al. 2013). The *tumor microenvironment* (e.g., the extracellular matrix²⁰, fibroblasts²¹, immune cells²², blood vessels) occupies a prominent role in the origin of intratumor heterogeneity. Interestingly, and with some ambiguity, its role is discussed either in passive terms, so that the cells maintain the relevant properties that are responsible for cells' invasion (Hanahan and Weinberg 2011), or attributing to the microenvironment permissive properties. In this latter case, the microenvironment seems to create, in fact, an abnormal context that favors stress responses, genetic instability and the selection of more invasive phenotypes (Polyak et al. 2009; Marusik et al. 2012; Spill et al. 2016; Lin and Lin 2019). In this sense, the tumor microenvironment may determine the epithelial-mesenchymal transition which is a process during which epithelial cells lose fundamental capacities such as cell-

¹⁷ p53 is a protein that prevents cancer formation, thus functioning as a tumor suppressor.

¹⁸ MicroRNAs are small non-coding RNA molecules, discovered in some viruses, plants, and animals, that are involved in RNA silencing and post-transcriptional regulation of gene expression.

¹⁹ CpG islands are DNA regions rich in cytosine and guanine. They can change their expression through methylation and, as such, they play an important role in epigenetics.

²⁰ The extracellular matrix is a three-dimensional structure consisting of extracellular macromolecules (e.g., collagen, enzymes, glycoproteins) the function of which is to provide surrounding cells with structural and biochemical support (Theocharis et al. 2016).

²¹ Fibroblasts are cells synthesizing the extracellular matrix and collagen.

²² Examples of immune cells are macrophages and lymphocytes.

cell adhesion and cell polarity and gain migratory properties that allow them to become mesenchymal stem cells; thus promoting cancer progression (Polyak and Weinberg 2009). The tumor microenvironment also promotes the vasculogenic mimicry²³ in such a way that tumor can transport material among each other; thus facilitating tumor growth (Hendrix et al. 2003). In general, the tumor microenvironment radically modifies the functional organization of healthy cells in such a way that new environmental niches appear: for instance, the abnormal vascularization of malignant tumor cells determines a very irregular vascular architecture with inefficient blood vessels with considerable fluctuations of oxygen over time (Marusyk et al. 2012). The development of tumor heterogeneity is also influenced by some micro-environmental factors such as exposure stress (e.g., anti-cancer drugs), tissue specificity, nutrition status, alterations of the physiological conditions (e.g. alterations in the hormone and metabolic levels) (Heng et al. 2009; McGranahan and Swanton 2017).

Beyond such interesting information about the role of the microenvironment, a fundamental assumption underpinning the SMT is that intratumor heterogeneity is an important cause of *tumor evolution*, because it would favor the formation of more aggressive and adapted tumor cells (Merlo et al. 2006; Heng et al. 2009; Polyak et al. 2009; Ye et al. 2009; McGranahan and Swanton 2017). Indeed, it has been argued that the mutations in the gene structure and in the number of gene copies that characterize intratumor heterogeneity are fundamental causes of the *microevolution* of malignant tumor cells, because they represent a raw material for selection to act on genes and they also permit the change in allele frequency, thus determining the appearance of new genetic variants (Heng et al. 2009). An indirect, though important, source of microevolution is represented by epigenetic mechanisms, which control gene expression in such a way that genes improve their adaptive capacities to the selection pressures of the environment. As such, epigenetic mechanisms play an important role in cancer progression and in the response to anti-cancer drugs (Marusik and Polyak 2009; Lin and Lin 2019). Likewise, tumor microenvironment promotes the selection of adapted cells by promoting different selective pressures on the same tumor, thus driving the evolutionary trajectories of tumor cells (Marusik et al. 2012; Gerashchenko et al. 2013). It is interesting to observe that in this context the term “evolution” could be a bit problematic, because the “natural evolution of cancer” refers to the development of the disease in *absence* of any *external conditioning*. Nevertheless, a population of tumor cells behave and “evolve” under the action of constraints, such as those placed by the microenvironment, the treatment, and artificial (laboratory) constraints. Thus, the term “natural evolution” in the context of cancer should be taken with a grain of salt.

²³ Vasculogenic mimicry is formation of micro-vessels by tumor cells.

According to the SMT, intratumor heterogeneity is a fundamental cause of cancer progression in *space* and *time* because of the role played by genetic, epigenetic and environmental causes. Indeed, nucleotide changes, modifications of chromosomes, and epigenetic mechanisms determine an overall genomic instability and environmental factors may favor multiple rounds of *proliferation* of the clones, usually accompanied by *cell death* (Marusyk and Polyak 2010). Moreover, the assumption of anti-cancer drugs may favor the appearance of genetic and phenotypic variants that promote cancer progression instead of inhibiting it (Swanton 2012; McGranahan and Swanton 2017). Compared to “natural evolution”, progression is a far more entangled process, as it depends from the combined interplay of a number of factors located at higher levels (tissues, organs, immune system and so forth) that constrain the progression of tumor cells.

In the light of the above, we can draw some important conclusions. First, SMT accounts share a *reductionist* account of tumor heterogeneity, because the relevant causes for understanding intratumor heterogeneity are identified with genetic and epigenetic mechanisms and only partially with the extracellular (or environmental) context. Secondly, the genetic, epigenetic, and environmental factors underlying tumor heterogeneity are important sources of the *microevolution* of malignant tumor cells. However, a population of malignant tumor cells can scarcely undergo macroevolution, because tumor cells independently change their genetic profiles, thus not generating a population with a homogeneous genetic set-up. Thirdly intratumor heterogeneity plays a pivotal role in cancer *progression*, because it favors the development of multiple rounds of proliferation and the invasion of other tissues.

3.2 Intratumor Heterogeneity from the Perspective of the TOFT and the Systemic Models of Cancer

A different explanatory perspective on intratumor heterogeneity has been provided by the TOFT and some systemic models in cancer research. The common denominator of these theoretical frameworks is the acknowledgement that the genetic and epigenetic levels of the cells do not satisfactorily explain cancer dynamics and tumor heterogeneity. Especially when the focus (*explananda*) is on carcinogenesis, they suggest that cancer is a multi-level disease, the biological features of which can be understood by examining the different (hierarchical) levels of organization of the overall organism and their functional inter-dependencies.

According to the TOFT, carcinogenesis is not caused by genetic mutations, but rather by some single or multiple carcinogenic exposure acts that interfere with the normal biophysical and

biochemical communication between the mesenchyme²⁴/stroma²⁵ and the parenchyma²⁶ in a given morphogenetic field²⁷ (Soto and Sonnenschein 2004, 2011). According to the TOFT, the “default” state of a cell is *proliferation* (and *motility*) (Soto and Sonnenschein 2004). The origin and progression of malignant tumor cells is due to a *loss of constraints* at the tissue level in such a way that the proliferative and motile capacities of tumor cells are no longer inhibited.

Although Soto and Sonnenschein’s theory does *not* give a *clear* account of the genetic and phenotypic *heterogeneity* of cancer, they seem to connect tumor heterogeneity with the disruption of the constitutive organization of the relationship between the parenchyma and the stroma. A correlation between tumor heterogeneity and an extensive alteration between parenchymal and stromal cells has been suggested by some research works (not necessarily adhering to the TOFT as an overall comprehensive theory about cancer) (Mueller and Fusening 2002; Shtilbans 2013). This is consistent with the observation that much of tumor heterogeneity is found in their stromal compartments and that both neoplastic cells and stromal cells around them change progressively during the multistep transformation of normal tissues into high-grade malignancies (Hanahan and Weinberg 2011).

Another important perspective on intratumor heterogeneity is provided by systemic models²⁸ in cancer research which study tumor heterogeneity by considering the way in which the cell changes its functional states (Heng et al. 2009). As pointed out by Heng et al. (2009), the genetic and epigenetic mechanisms underlying tumor heterogeneity do not make sense if separated by the dynamics of the functional states of the cellular system.

From a systemic perspective, *tumor microenvironment* plays a pivotal role, in explanatory terms, both in tumor progression and in intratumor heterogeneity, since it is the biochemical *support* of the *morphogenetic field*, which drives epithelial cells towards differentiation and phenotype transformation (Nelson and Bissell 2006; Bizzarri and Cucina 2014). A deregulation in the balance between morphostats²⁹ and morphogens³⁰, for example, would lead to further abnormalities in cell proliferation and tissue organization (Bizzarri and Cucina 2016). Intratumor heterogeneity would be the outcome of the complex cross-talk among stromal cells, epithelial cells, and tumor microenvironment: the interactions between epithelial cells and microenvironmental components (particularly stromal cells) change the extracellular matrix composition as well as its biochemical-

²⁴ The mesenchyme is a type of connective tissue mostly found during the embryonic development of bilateral animals.

²⁵ The stroma is a part of a tissue (or organ) with a connective role.

²⁶ The parenchyma is the functional part of an organ.

²⁷ A morphogenetic field is a group of cells involved in the morphogenesis of tissues and organs.

²⁸ An in-depth discussion of the systemic models in cancer research can be found in Bertolaso (2016, pp. 43-59).

²⁹ Morphostats are molecules, mostly produced by fibroblasts and macrophages, that regulate cell proliferation.

³⁰ Morphogens are molecules that govern the pattern of tissue development in the process of morphogenesis.

biophysical features (Bizzarri and Cucina 2014). The change in the tumor microenvironment entails a structural and functional reorganization of the overall cell in such a way that the genetic and phenotypic changes of tumor cells (including their heterogeneity) must be understood in the light of this *cell-tissue continuum* (Nelson and Bissell 2006). For instance, it has been observed that alterations in the relationship between cytoskeletal filaments and proteins and the extracellular matrix have far-reaching consequences not only for the cell shape and tissue modeling, but also for the enhancement of cell proliferation and migration (Ingeber 2008; Bizzarri et al. 2015). It is widely accepted that cancer dynamics should be studied by considering both the tumor microenvironment and the “tumor organismal environment”, which includes the immune system, the nervous system and the microbiome (see Laplane et al. 2019).

To conclude, the TOFT and the systemic models underline the importance of the *cell-tissue continuum* to understand the origin, the progression, and the tumor heterogeneity of malignant tumor cells. This implies that the primary causes of tumor heterogeneity do not lie in genetic and epigenetic mechanisms, but rather in an overall structural and functional reorganization of the cell-tissue interactions that determine genetic and epigenetic mutations. Hence, the relationship between microenvironment and tumor cells is no longer a mere co-factor, but rather the constitutive context of tumor heterogeneity and cancer progression.

4. Tumor Heterogeneity as a Complex Phenomenon

The SMT and the systemic accounts³¹ have differently explained tumor heterogeneity, considering it as the result of either genetic and epigenetic modifications or alterations at the tissue (and organ) level. In spite of this major difference, do these theoretical perspectives provide us with a *common* core concept of complexity of tumor heterogeneity? In order to answer to this question, we consider some features of tumor heterogeneity on which both accounts agree, despite somehow the different explanatory accounts: first, the relevance of *genome instability*; secondly, the role played by *tumor microenvironment*; thirdly, tumor heterogeneity as a fundamental feature of *tumor progression*; fourthly, the influence of tumor heterogeneity on the *spatial and temporal organization* of a metazoan; finally, tumor heterogeneity as a causal related aspect of the *loss of systemic regulation*.

A first convergence point between the SMT and the systemic accounts lies in recognizing *genome instability* as a major driver of tumor heterogeneity. Genome instability is a broad term referring to the high frequency of genetic mutations in a genome (e.g., changes in DNA sequences, chromosomal rearrangements, appearance of an abnormal number of chromosomes) that are at the origin of the continuous appearance of new genetic and phenotypic traits, and therefore *intratumor*

³¹ Hereinafter, we use the term systemic accounts to refer both to the TOFT and to the models of systems biology.

heterogeneity, during cancer initiation and progression. Although the SMT and the systemic accounts agree that genomic instability is a fundamental cause of intratumor heterogeneity, they differently explain it³²: according to the SMT, genome instability is mostly caused by random alterations at the gene level (e.g., modifications of DNA repair mechanisms) (Swanton 2012; McGranahan and Swanton 2017); the systemic accounts, instead, interpret genomic instability as the outcome of systemic alterations (e.g., alterations in the paracrine signals expression that lead to a failure in intercellular communication or the destruction of the extracellular matrix) (Nelson and Bissell 2006; Bizzarri and Cucina 2014).

There is a consensus between the SMT and the systemic accounts about the role played by *tumor microenvironment*, inasmuch as both theoretical perspectives agree that genetic and phenotypic heterogeneity are affected by the components of the extracellular environment (e.g., the extracellular matrix, fibroblasts, blood vessels). The SMT and the systemic accounts emphasize different but not mutually exclusive aspects of tumor microenvironment. According to the former, the tumor microenvironment provides tumor cells with a *structural support* and *nutrients*, thus determining an abnormal context that favor stress responses, genetic instability and the selection of more invasive phenotypes (Spill et al. 2016; Lin and Lin 2019). The latter considers tumor microenvironment as a *physical-chemical constraint* that drives the differentiation and the genetic and phenotypic transformation of epithelial cells (Bizzarri and Cucina 2014). Modifications in the tumor microenvironment (e.g., in the architecture of fibroblasts or extracellular matrix) trigger alterations in the gene expression of paracrine signals and their extracellular release, thus affecting intercellular communication and the coordination among the cells of a tissue which may in turn affect genome instability and the production of aberrant phenotypes (Bizzarri and Cucina 2014; Baghban et al. 2020; Brassart-Pasco et al. 2020). This difference is substantial in explaining tumor heterogeneity: for the SMT tumor microenvironment is a factor *promoting* tumor heterogeneity, but it has no priority over the genetic mutations; by contrast, the systemic accounts consider the modifications occurring in the tumor microenvironment as a *primary cause* of the appearance of intratumor heterogeneity.

Both the SMT and the systemic models reject the idea that intratumor heterogeneity is a *mere* stochastic molecular noise determining random variations in gene expression levels; in fact, these two theoretical perspectives recognize that, despite its randomness, the emergence of genetic and phenotypic heterogeneity is a *systematic cause* of *tumor progression* and, as such, it must be regarded as a *systemic property* of the pathophysiology of cancer. According to the SMT, the appearance of genetic heterogeneity in tumor cells represents a raw material upon which selection

³² As pointed out by one referee, these views are not mutually exclusive.

can act, thus determining the appearance of new (more adapted) genetic variants (Heng et al. 2009; Gerashchenko et al. 2013). The microevolution of tumor cells in turn favors the progression of cancer, inasmuch as tumor cells can respond to stress factors (e.g., anti-cancer drugs) more effectively (Heng et al. 2009). In the systemic models, the progression of cancer is enabled by the structural and functional changes in the tumor microenvironment and the appearance of genetic and phenotypic heterogeneity (Bizzarri and Cucina 2014).

Another convergence point between the SMT and the systemic models is the idea that intratumor heterogeneity alters the *functional integration* of cells, tissues, and organs of a metazoan. The reason is that the emergence of new genetic and phenotypic traits modifies the normal *spatial organization* of anatomical parts, thus interfering with the way in which the functions performed by these parts are integrated among each other. For example, an alteration in the gene expression of signaling pathways may alter epithelial cell polarity as well as cell size and shape (Etienne-Manneville 2008; Lee and Vasioukhin 2008). Furthermore, tumor heterogeneity affects the *temporal coordination* between the functions performed by different anatomical parts, thus determining a decoupling among them. For instance, the introduction of genetic and phenotypic variants in growth factor pathways may determine a loss of temporal coordination with metabolic processes, leading to an abnormal proliferation of tumor cells (Sever and Brugge 2015).

All in all, both the SMT and the systemic models recognize that intratumor heterogeneity produces an *inter-level dysregulation* during which the signaling and regulatory pathways underlying cell differentiation, apoptosis, and tumor proliferation become *uncoupled*, the relationship between the stroma and the parenchyma is radically altered, and organs are seriously compromised. As a result, the normal functional integration and the hierarchy of cells, tissues, and organs are progressively lost, thus undermining the systemic stability. The loss of a hierarchical organization during cancer initiation and progression is accompanied by the appearance of *aberrant* and *unorganized* genetic and phenotypic heterogeneity which in turn upsets the proper balance between homeostasis and the maintenance of *ordered* functional heterogeneity (Bertolaso and Dupré 2018).

Now, what can we infer from the above-mentioned features to understand the complexity of tumor heterogeneity? In our view, tumor heterogeneity is *complex* inasmuch as a) it occurs at *different levels* involving *heterogeneous entities*; and b) these entities are *causally interdependent*, thus establishing an intricate network of causes and effects. Let us argue this salient point.

Tumor heterogeneity occurs at different levels because it is the *cause* of (e.g., the loss of spatio-temporal integration among the levels of a metazoan, the failure of systemic regulation) and, at the same time, the *effect* of (e.g., genomic instability, alterations in the tumor microenvironment) several dysfunctionalities encompassing different entities (e.g., nucleic acids, proteins, cells,

tissues) at the different organizational levels (i.e. genes, cellular network, cell-cell interactions, tissue, organs, and organ systems) of a metazoan. As a result, the overall functional integration of a metazoan is compromised. Tumor heterogeneity entails an extremely *intricate network of causal relationships* where three thorny problems arise, all having far-reaching consequences for the explanation of tumor heterogeneity throughout cancer progression.

First, it is hard to sharply distinguish between the *primary* (or direct) and the *secondary* (or indirect) causes (if any) involved in the appearance of new genetic and phenotypic traits. The controversy between the SMT and the systemic accounts over the factors causing tumor heterogeneity (and carcinogenesis as well) is indicative of the difficulty of establishing which factors are primary or secondary causes of tumor heterogeneity. Apparently, it seems that genetic and epigenetic factors are as primary causes as systemic alterations (e.g., modifications in the parenchyma-stroma interactions), thus giving the impression that each organizational level is at the same time relevant to understand the emergence of tumor heterogeneity. This entails a disagreement over the more appropriate pharmacological treatments of tumor heterogeneity (and hence tumor progression): on the one hand, SMT accounts endorse a pharmacological approach that targets the proteins synthesized by oncogenes (e.g., trastuzumab targets the protein HER2 which is synthesized by the oncogene *HER2*); on the other hand, systemic accounts stress the importance of differentiated pharmacological therapies that target not only the genetic level but also the cellular and microenvironmental contexts (e.g., manipulation of cancer metabolism) (Yoshida 2015; Bizzarri et al. 2020a).

Secondly, a corollary of the difficulty of establishing a clear hierarchy of causes of tumor heterogeneity is that it is very problematic to exactly determine their *temporal dependence relations* (i.e. diachrony or synchrony, and what kind of reflexivity holds). This is manifest when we consider, for example, the temporal causal relation between genetic alterations, genomic instability, and tumor microenvironment: in spite of being considered causes of tumor heterogeneity, it is very difficult to determine the *exact* causal relation among them in terms of *temporal dependence*.

Finally, the lack of a consensus on the primary and secondary causes of tumor heterogeneity and on their temporal order poses great challenges to the interpretation and integration of *empirical data*. For example, since the (temporal) order of the causal relationships between genetic mutations, the alterations of regulatory, signaling, and metabolic pathways underlying intratumor heterogeneity is not well known, cancer research copes with a considerable difficulty in interpreting and integrating the empirical data coming from genomics, transcriptomics, proteomics, and metabolomics (Plutynski 2013; Boniolo and Campaner 2019).

5. Pharmacogenomics in the Light of the Complexity of Intratumor Heterogeneity

After having examined the organizational levels of tumor heterogeneity and its complexity, let us assess the impact of genetic and phenotypic heterogeneity on the epistemological framework and the scientific practice of pharmacogenomics, in order to understand the limits of this branch of personalized medicine and outline some strategies to cross them, thus revealing the potentialities of pharmacogenomics in cancer treatment.

The rationale behind pharmacogenomics is that the effectiveness of drug metabolism hinges on the genetic variations responsible for a certain disease. Accordingly, the main purpose of pharmacogenomics is the discovery of genetic (e.g., nucleotide polymorphisms or variations in the copy number of genes) and genomic variations (e.g., variations in the copy number of genes) behind a disease in order to understand how these modifications affect pharmacokinetics, pharmacodynamics³³, and immunological responses, and therefore to find the correct dosage of a drug and its gene targets (Crews et al. 2012). Such a gene-based approach to pharmacology relies on the implicit assumption that (acquired or inherited) gene variations occur only *once* and that they do *not change* during a pathophysiologic process.

Nevertheless, what tumor heterogeneity teaches us is that cancer initiation and progression are characterized by a continuous change both in single genes, in allele frequency, and in the overall genome, leading to dramatic changes in the gene expression and phenotypic features of tumor cells. Furthermore, genetic variations are neither the only nor the principal causes of tumor heterogeneity, because many other modifications in the regulatory, signaling, metabolic pathways, as well as in the tissue and organ architecture play a fundamental role in cancer initiation and progression. This being the case, the epistemological premise of pharmacogenomics is seriously compromised because of a *continuously* changing genome that entails the *constant* appearance of new phenotypic variations and, therefore, new dynamics in the metabolism of drugs.

The weakening of the epistemological support of pharmacogenomics in cancer treatment is reflected in the limits of its *diagnostic* and *predictive capacities*. Since tumor heterogeneity is a major source of variability in tumor cells, a question arises as to whether a single sample from one specific tumor region is representative of the whole corresponding tumor. Regardless of the selection criteria, the analysis of a single sample reveals some genetic and phenotypic features that are unique to that single sample, and therefore not representative of the tumor as a whole (Cyll et al.

³³ Pharmacokinetics is the study of how an organism affects a drug (e.g., drug metabolism and elimination), whereas pharmacodynamics is the study of how a drug affects an organism. Pharmacokinetics and pharmacodynamics are the main branches of pharmacology,

2017). A further problem is posed by pharmacogenomic biomarkers³⁴, the discovery of which relies on genetic or transcriptomic analyses of single tumor biopsies taken from primary or metastatic lesions. Genetic and phenotypic heterogeneity may produce *biases* in tumor samplings and in the validation of biomarkers, because it may suggest incorrect causal relationships between (heterogeneous) genetic alterations and clinical outcomes in the discovery phase (Swanton 2012). For example, it has been shown that intra- and inter-tumor heterogeneity preclude the use of some predictive biomarkers (e.g., Prolaris and Decipher) on a single biopsy to administer a successful treatment (Cyll et al. 2017). Accordingly, as pointed out by Plutynski (this volume), the informational value of cancer biomarkers is rather limited and this reduces their ability to predict the risk of recurrence or to make decisions about treatment.

As a further consequence of the epistemological fragility of pharmacogenomics determined by tumor heterogeneity, the *prognostic power* (and the clinical effectiveness) of pharmacogenomics is undermined. On the one hand, pharmacogenomics has produced positive results in cancer treatment, because the progression of many solid tumors has been shown to be slowed down by identifying and targeting some gene variants for receptor tyrosine kinases (e.g., EGFR, ERBB2, JAK2) (Lauschke et al. 2018). On the other hand, tumor heterogeneity causes additional mutations that lead to drug resistance “even in patients that are initially responsive to targeted therapy” (Lauschke et al. 2018, p. 8). Although some malignant tumors (e.g., chronic myeloid leukemia) have successfully been treated by inhibiting tyrosine kinases (e.g., by employing the imatinib), many anticancer drugs are mutagens that could potentially cause *de novo* drug-resistance mutations (Lauschke et al. 2018). The mutagenesis produced by anti-cancer drugs is due to the general genome instability of neoplasms, which is closely connected to the genetic and phenotypic tumor heterogeneity. Thus, although a number of anti-cancer drugs have been shown to improve the progression-free survival, they do not offer an overall survival benefit to patients (see Plutynski, this volume).

The main limitations of pharmacogenomics in the treatment of tumor heterogeneity and cancer progression can be understood in the light of a general theoretical paradigm that dominates the current pharmacology: the idea that *one* drug binds to *one* molecular receptor in order to cure *one* disease (the so-called one drug-one target-one disease approach). Such a theoretical perspective considers the molecular level (genes and receptors) as the most explanatorily relevant one, thus overshadowing other potentially relevant organizational levels (e.g., tissues) for the study and

³⁴ Biomarkers are measurable indicators of some biological state which can have a predictive, a diagnostic, or a prognostic value. In personalized cancer medicine, pharmacogenomic biomarkers are mutated genes (found in tumor cells) that are considered explanatorily relevant for the prediction and diagnosis of cancer, and for the identification of new potential therapeutic targets (see Plutynski, this volume).

development of drugs. So that, pharmacogenomics is the product of such a conceptual framework, hence suffering from the same epistemological and practical limitations. It therefore seems that a new and perhaps more fruitful way to conceive pharmacogenomics demands a *change* in the *paradigm* of pharmacology: the transition from a *molecular-* to a *system-*based pharmacology in which the molecular level is not necessarily the most relevant one to drug discovery and action, but rather one of the several interconnected levels that underlie pharmacokinetics and pharmacodynamics (Bizzarri et al. 2020b). Let us address this fundamental point, which represents the *pars construens* of our argument concerning pharmacogenomics.

There are two related senses in which we can talk about a system-based pharmacology. First, the idea that pharmacokinetics and pharmacodynamics can be studied through the construction and the analysis of *network models* that study how different functional entities involved in drug response (e.g., transcription factors, enzymes, signaling molecules) interact among each other *over time* (Danhof 2016). Secondly, the view that *inter-level* relationships and *systemic* properties take priority over gene and molecular pathways, not in the trivial sense that the molecular level is not important, but rather that it (as well as the other organizational levels) is entirely dependent on the inter-level relationships and systemic properties³⁵ (Bizzarri et al. 2020b) because of a different causal dynamics that take place *in time* and that justify the epistemological relevance of adopting the natural history of the organism as a privileged viewpoint (Bertolaso 2016; Bertolaso and Dupré 2018).

Now, to what extent a system-based pharmacology could be a fruitful tool for the treatment of tumor heterogeneity and cancer progression? A first potential benefit of systems pharmacology is its *predictive* and *diagnostic power*, as it describes the effects of dynamic complex patterns of drug action (e.g., oscillatory patterns) on disease progression (e.g., the exponential progression patterns of cancer). Systems pharmacology studies pharmacokinetics and pharmacodynamics as *dynamical* systems, the evolution of which is predicted by solving a set of differential equations (Danhof 2016). Thus, since tumor heterogeneity is a dynamic process that enables tumor cells to develop new ways to respond to anti-cancer drugs, systems pharmacology could be extremely valuable to make more accurate predictions of how anti-cancer drugs are metabolized and which effects they may produce throughout cancer progression.

A second advantage of system-based pharmacology in cancer treatment is its *prognostic* value, because it seeks to target some entities and mechanisms that are at the root of *tumor heterogeneity* in at least three ways. First, whereas a molecular-based pharmacology develops drugs that target a

³⁵ This view is ontologically grounded on a relational view (e.g., Bertolaso 2016; Bertolaso and Dupré 2018) and on an organizational account (e.g., Mossio et al. 2009) of biological functions.

single gene (or protein), the system-based one adopts a multi-target approach, thus opening up the possibility of using *new therapeutic strategies* also based on nonconventional mechanisms of action (Bizzarri et al. 2020b). For example, nonconventional strategies, such as foams and creams, have been starting to be employed in the cancer treatment in order to promote hydration, inhibit contractile tension, and reduce the local production of reactive oxygen species, thus reestablishing the normal epidermal barriers (Bizzarri et al. 2020b). Secondly, special emphasis is put on *homeostatic mechanisms* that can be exploited to control cellular states. For instance, it has been suggested that epithelial homeostatic mechanisms can be harnessed in order to foster the elimination of precancerous neighbors by epithelial cells (Lahvic and Hariharan 2019). Thirdly, there is an increasing effort to study *tumor microenvironment* with the tools of the computational models of systems pharmacology. For instance, there already exists a computational model that studies the temporal evolution of selected factors associated with immunoactivation or immunosuppression of the tumor microenvironment in order to improve cancer immunotherapy (Mpekris et al. 2020). All in all, these therapeutic strategies likely have the potential to undermine some fundamental (systemic) causes of tumor heterogeneity.

If considered in the context of a system-based pharmacology, pharmacogenomics gains a new *diagnostic* and *prognostic* significance. Indeed, if tumor heterogeneity can be blocked or, at least, slowed down by using a dynamic multi-target approach, then pharmacogenomics turns out to be useful to investigate which *genes* may interfere with or undermine the metabolism of anti-cancer drugs and to find the *correct dosage* of them. In this respect, the employment of pharmacogenomics makes sense only *after* that tumor heterogeneity has successfully been stopped (or slowed down) and always in synergy with systemic, and not only gene-based, pharmacological approaches to cancer.

6. Concluding Remarks

In the previous sections, after having explored the levels of organization of tumor heterogeneity from the perspectives of both the SMT and the systemic accounts, we have examined some characteristics of tumor heterogeneity that make it a *complex* phenomenon. We have argued that the complexity of tumor heterogeneity lies in that it is a nested phenomenon occurring at different organizational levels and exhibiting an intricate network of causal relations. Such a complexity has important consequences not only in the context of scientific research, but also in the clinical pharmacological treatment of cancer. Indeed, the genetic and phenotypic heterogeneity of tumor cells significantly weakens the epistemological premise of pharmacogenomics based on the idea that gene variations occur only once and that they do not change over time. This epistemological

fragility entails important limitations in the diagnostic, predictive, and prognostic capacities of pharmacogenomics.

We have argued that these epistemic and practical problems of pharmacogenomics are due to the epistemic limitations of the theoretical paradigm of classical pharmacology, which does not assess the complexity of systemic responses to drugs. Therefore, we have suggested that a potential fruitful way to conceive pharmacogenomics is within the theoretical framework of systems pharmacology which opens up the possibility of more accurate predictions of the dynamics of anti-cancer drugs and new (multi-target) pharmacological strategies for tumor heterogeneity and cancer progression. In the context of a system-based pharmacology, there are more concrete possibilities for successfully treating tumor heterogeneity and pharmacogenomics can be a useful tool for finding the genes affecting drug metabolism and for discovering the correct dosage of anti-cancer drugs.

We may now wonder which are the future challenges of pharmacogenomics in cancer treatment and what practical consequences could ensue from our thesis. First, a systemic framework for the pharmacokinetics and pharmacodynamics of anti-cancer drugs entails the challenging task of developing new computational models for the integration and interpretation of different data. Secondly, a biological and philosophical study on the concepts underlying the biological organization of cancer as a whole (e.g., functional integration, regulation, evolvability) will be required. Thirdly, translational research in personalized medicine will likely attract new public and private investments in order to convert the results of the systemic (computational) models of cancer into fruitful anti-cancer treatments. Finally, personalized cancer medicine would eventually relativize the (alleged) deterministic power of genetic mutations in favor of a systemic view of cancer. This may psychologically help oncological patients to consider cancer not as an inherited and inescapable disease, but rather as a very complex pathology that, if treated in a systemic way, could be cured.

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