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STATIC VS DYNAMIC MODELS IN CANCER RESEARCH TODAY:

new or unanswered questions which still challenge our modeling and understanding of cancer.

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ABSTRACT:

Today's inconsistencies in our cancer understanding are here discussed with the aim of improving modeling and relative strategic and therapeutic options in the future. Five simple unanswered questions were enucleated: 1. Peto's Paradox (PP), which despite its long-lasting discussions and some newly discovered molecular mechanism, remains unexplainable for several log.s (9-12) of cancer risk; 2. the low spontaneous mutation rates relatively to today's accepted multistep (10-12) models, thus suggesting additional mechanisms of mutagenesis or/and carcinogenesis; 3. some mechanism of adaptive mutations indicated by seminal work of John Cairns and others but never fully clarified; 4. the relative role of selection versus variation (mutation) in cancer cell evolution; 5. illuminating new discoveries are emerging from studies on species with extreme longevities (20-30 X). They also suggest alternative mechanism(s) of carcinogenesis or resistance to it. A final overview is made of cancer modeling itself as epistemological instrument; here: I) Popper's falsification principle should be also applied –for example by analyzing upstream and downstream levels of carcinogenesis- as well as to therapies based on the underlying model (i.e., *TGT*s) and II) Kuhn's paradigm-shifts could be predicted according to the several model-anomalies and therapies-failures (for cancer complete-cure). An additional shift is suggested in considering several potential models at the same time, instead of just the prevailing one: 10 models were deduced from the current work in cancer research. Further distinction can be made between Static vs Dynamic Models. The first ones tend to describe the Cancer Landscape, sometimes in the greatest molecular/cellular detail. Dynamic models instead emphasize the underlying mechanism(s) or "cancer engine" for initiation, progression and tumor heterogeneity. Finally, Bivalent Models consider both Static and Dynamic mechanisms in different cancer types/states. Heuristic value will be finally determined by experiments and deduced therapeutic applications with a strategy of accelerating discoveries and cures.

Introduction.

Understanding the origin of a dreadful disease such as cancer has been a logical and legitimate aspiration of *H. sapiens*, since the time of ancient Greeks with Hippocrates and

Aristoteles [1-2], Romans with Galen [1, 3] or the scientific Renaissance with Vesalius [4-5]. The great emphasis of cancer research was initiated by the German school of R. Virkow and colleagues, but has seen its golden age after Molecular Biology birth date (1953) [6]. Starting with the great accomplishments in 1970's-90's, molecular details of the neoplastic cell and of the carcinogenic process have been dissected. The vast and ever growing amount of molecular biology data on cancer cells has been condensed in the *"Hallamarks of Cancer" (HoC)* model by Hanahann and Weinberg initially published in 2000 and revised in 2011 [7-8]. The recent text "The Biology of Cancer" by R. Weinberg could be considered the "Summa" of our present molecular and cellular understanding [9]. However, the picture proposed in *(HoC)* appears today insufficient to answer many of our questions on cancer [6, 10]. This brief opinion paper and review does not want to provide definitive answers about today's cancer research unresolved issues. Rather, it wants to propose an overview of some of the most challenging questions and directions for future research [10].

I. **Previous Models as methaphor.** I will therefore emphasize open questions rather than provide definitive answers and what such open questions imply for our understanding of cancer. Interestingly, this manuscript was largely inspired by a non-scientific text, which overviews the cancer understanding throughout Centuries: "*The Emperor of all Maladies*" by Siddhartha Mukherjee [11]. The several leads followed by *H. Sapiens* in order to explain cancer become here a kind o *metaphor* for cancer research, with possible implications even for our present understanding . Just to provide an example well described in the 5-700 pages of Mukherjee's text: for approximately 80 years, women with breast cancer were extensively amputated following the recommendations for "radical mastectomy" of William Halsted (one of- if not the most regarded oncologic surgeon in history) and his school [12-14]. It is acknowledged today - thanks to the work of B. Fisher, G. Canellos, G. Bonadonna and many others [15-19] - that this practice was excessive and in fact wrong. Similarly, it is recognized that the underlying model, the "centrifugal" model of William Handley, was incorrect [20-21]. It is however interesting to observe that falsification of Halsted model and practice (I will come back to the *falsification* principle by Popper in Section III [22]) was achieved first through the mentioned clinical studies. The metaphor however is apparent: our strategic and therapeutic implementation for patients treatment starts with an underlying model in order to explain cancer, certainly not a trivial pursuit. Furthermore, our models could be wrong today as they were at the time of Halsted (*Radical-Surgery Model*) [23] or Galen or Vesalius (*Black-Bile Model*) {, 2009 #16312}. Unquestionably, today's model most quoted in the literature and *tout-court* most accepted is the above mentioned *HoC* (2000 and 2011). However, as discussed in III, the therapies derived as logical consequences from *HoC* seem to be insufficient or incomplete (see also [6, 10]). Furthermore, there is a growing number of questions that *HoC* (or other models) is unable to answer or explain: these will be discussed in 5 points as it follows in II.

II. **5 simple questions still unanswered by *HoC* or cancer research in general:**

Fig. 1 5 simple questions still unanswered by *HoC* (or cancer research in general)

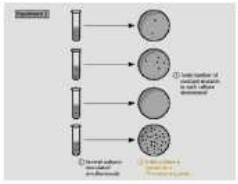
Unanswered Question	Relevant publications
1 Richard Peto's Paradox (PP)  Sir Richard Peto, FRS	{Peto, 1975 #15220} {Peto, 1997 #16285} {Peto, 2015 #16283} {Nunney, 2015 #16287} {Caulin, 2015 #16314} {Boddy, 2015 #16316} {Abegglen, 2015 #16327} {Sulak, 2016 #16328}
2. Low Mutation Rates 	{Luria, 1943 #8931} {Drake, 1998 #16330} {Baer, 2007 #16338} {Jobling, 2017 #16339}) {Kunkel, 2000 #16340}
3. Darwinian vs Adaptive Mutations   Darwinian vs Adaptive Models	{Cairns, 1988 #16291} {Cairns, 1991 #16290} {Rosche, 1999 #16361}. {Rosenberg, 2001 #16349} {Rosenberg, 2004 #16350} {Maisnier-Patin, 2015 #16292}
4. Mutation vs Selection 	{Tomlinson, 1999 #8906} {Peto, 2001 #7445} {Tomasetti, 2015 #15899} {Rovigatti, 2015 #15855} {Luzzatto, 2015 #16281}
5. Species with Extreme Longevities   Naked Mole Rat Blind Mole Rat	{Seluanov, 2018 #16288} {Tian, 2018 #16289} {Lagunas-Rangel, 2018 #16373} {Sahm, 2018 #16372} {Gatenby, 2011 #11055} {Gatenby, 2012 #14692}

Figure 1 Legend: Five relevant question still unanswered by current modeling (HoC or others) are presented and discussed in the text and here briefly summarized with small iconography. 2 refers to the fluctuation test by Luria-Delbrueck [45] ; 3 shows Charles

Darwin and John Cairns; 4. cartoon relates to the paper by Tomlinson and Bodmer:" *…ensuring that the tail does not wag the dog…"* [81].

Question 1. ***Peto's Paradox, PP.*** Are mutations and mutation rates necessary and sufficient causes of cancer onset, in view of the well-known paradox associated to Richard Peto (*Peto's Paradox, PP*) [24] ? As Peto humbly described it, this could be also defined as Doll's dilemma or Cairns' conundrum: a logic question discussed for at least 7 decades, especially by scientists in UK [25] at least initially. Although it also deals with carcinogenesis and aging (see also question 5), *PP* is generally recognized as the constant/invariable cancer incidence through different species with very variable sizes a and longevities . Organisms which have been often considered in these studies are mice, humans and whales [25-26], where there is approximately 3000X variation in size (i.e. from 20 g in mice to 65 kg in humans to approx 200 ton in whales). Furthermore, while mice live approximately 2.5 years, both whales and humans show higher longevity (80 years). The generally acknowledged lack of variation in cancer incidence therefore requires an adjustment of several parameters of cancer risk. This is given by the formula Ct^6, meaning that it is proportional to a Constant C (determining susceptibility of cancer for that species) multiplied by time at the sixth power. However, since humans and whales are much larger than mice (3000 X and 10^7 X respectively) and both show approximately 30 fold more longevity (from 2.5 to 80 years), the formula is corrected by a factor of billions/trillions to show the much lower susceptibility/ gram of tissue and throughout lifespan [25]. In other words, we have to consider –inside a human or a whale- the many fractions of body with the size of a mouse and similarly reduce the lifespan risk for cancer (otherwise, in the presence of such multipliers, the calculated values in both humans and whales would be skyrocketing). One could somehow disagree with some of the calculations (as I do) between the papers of R. Peto himself and those in which he is co-author with the late R. Doll, but the bottom-line is unavoidable: this factor is very large [24-26]. For example, in Peto and Doll BMJ 1997 [26], approximately a trillion: from $10^{-7} \times t^5$ in mice to $10^{-19} \times t^5$ in humans. Therefore, *PP* describes this extremely high value of missing risk, refers to today's absence of a scientific meaningful and -most of all- *general* explanatory mechanisms and questions and challenges current science (with many more tools available today from molecular biology and genetics, so called *molecular oncology* [27] to provide acceptable solutions to the paradox. For example, a recent issue of the *Royal Society Philosophical Transactions* was dedicated to Peto's Paradox [28] with several possible explicatory mechanisms: 1. cross-species gene analysis and acquisition of increased onco-suppressive functions [29]; 2. cooperation vs. cheating in multicellularity [30]; 3. reproductive trade-off's [31]; 4. maternal fetal conflicts [32]; 5. inclusive fitness [33]; 6. life-history models [34] and [35]; 7. stem cells numbers and replication rates [36]; 8. the Hallmarks of Cancer (*HoC*) [37]; 9. cellular metabolism [38]; 10. infection responses [39]; 11. human cancers specific mechanisms [36] and 12. several examples from the field of comparative oncology [40-41]. However, even the introductory note by Richard Peto himself warned the reader [25] that the solutions presented in this collection (and many more retrievable through Medline) fall short (and greatly so !) to explain the vast differences of value in risk

per gram of tissue –according to *PP* -, which is evaluated in billion or even trillion folds (for example between mice and whales).
In this opinion-review however, I mostly focus on the impact of *PP* on our current cancer-modeling. In this view, *PP* simply contradicts the most accepted *CAN-GEN* models (such as *HoC*), by falsifying the premise of random mutations in specific gene families (*hallmarks*) proportional to size and longevity [6, 10]. Therefore, the current attempts of discovering novel means of evolution or tumor suppression for counteracting *PP*, such as the increase in *TP53* gene copy numbers in *proboscidates* [29, 42-43] is rather indicative of a "*paradigmatic science*", which, once anomalies or paradoxes are discovered within itself as theory or paradigm, tries to resolve them with tools inherent and contained in the same theory or paradigm. This is a well-known epistemological error described and studied by Thomas Kuhn in his *The Structure of Scientific Revolutions* (TSSR) [44] and will be discussed in section III (*Sci-Rev Model*).

Question 2. Low mutation rates. In view of the well-known low incidence of spontaneous mutations, how can such 10 or more mutational steps predicted by *HoC* and similar models (i.e., *Vog-2013*), be generated through life-span of *H. sapiens* (but also other species with higher longevity) ? The *fluctuation test* was projected by S. Luria and M. Delbrueck in 1943, in order to test the origin of somatic mutations [45], one question which had been already debated for at least one Century, for example by the work of August Weissmann (*L-D Model*) [45-46]. Here the selected trait/mutation was resistance to bacteripophage *T1* infection (*Ton*^{r}). The final calculations of mutation rates/ bp / cell division is performed through the Poisson Equation: $Px = \frac{h^x \times e^{-h}}{X!}$ where *h* is the average mutation rate after *N* cell divisions. Solutions were obtained with x = 0, in order to simplify experimental setting (since in several experiments there were many plates with 0 colonies of mutants/resistants). Therefore: $Px_0 = e^{-h} \rightarrow Log(Px_0) = -h$ *or* $h = -Log(Px_0)$. With experimental calculations, it is possible to obtain the total number of cell divisions through Tartaglia/Pascal's Triangles and the average mutation rate/cell division as approximately 3×10^{-8} [45]. Similar values have been then calculated for eukaryotic cells, also with additional experimental settings and more recently by DNA sequencing [47-49]. Data on mutation rates of eukaryotes and especially *Homo sapiens* derive from a compendium of studies: 1. studies on deleterious mutations, particularly illuminating in Alpert Syndrome and in *MEN-2A* and *MEN-2B* [47, 50]; 2. studies on hemoglobin genes changes [47, 51]; 3. studies on pseudogenes: these are important since such mutations are theoretically neutral and therefore not under the control of selection (85% of *Hb* pseudogene mutations are confirmed not to be associated to selection bias) [48, 52]; 4. starting from 2011, a series of important studies have been focused on assessing mutation rates through NGS studies of trios (2 parents and proband) or population analyses for well characterized *STRs* or *SNPs* [53-55]; 5. Y chromosome sequencing studies should be also mentioned, since they were/are at the forefront of several sequencing efforts, taking the advantage of a limited coding/expressed sequence, the so called MSY (male specific region of the Y chromosome), sometimes referred also as NRY (non-recombining region of the Y) [49]. Several biases were apparent and in part clarified in the past few years, such as 1. the above mentioned mutation-selection effect [47-48]; 2. the clear patrilineage origin of the majority of mutations [47,

53] and 3. the age association of mutation accumulation in males, so that -on average- approximately 20 mutations will be added in sperm every 10 additional years of paternal age [55]. These biases notwithstanding and although it would be difficult here to review an extensive body of data on *H. Sapiens* (but see the excellent reviews [47-49]), today's evidence converge toward an agreed value of approx. 1-2 x 10^{-8} Mutations/ per site/ per generation.
This very low value of mutation rates is however also confirmed by studies in the opposite direction, i.e. by measuring fidelity of the replication process: the major replication polymerases (*pol III* in pro- and pol **ε/δ** in eu-karyotes) assure a very low rate of mis-incorporation, approximately 10^{-3}) [56], this is usually corrected by an ad-hoc proofreading pocket (10^{-2}, i.e. $10^{-3}\rightarrow 10^{-5}$) [56-57] and by an additional powerful post-replicative correction performed by Mis-Match Repair (MMR, see Paul Modrich's work [58-59]), MMR (10^{-3}, i.e. $10^{-5}\rightarrow 10^{-8}$) [57, 60].
Such low mutation rates create a logical problem also in understanding carcinogenesis, according to the most accepted/discussed model, i.e. *HoC* or similar multi-step models: rapid calculations for the different models provide very low values: *HoC* 2000 → 6.4 x 10^{-47}; *HoC* 2011 → ≈ 10^{-77} ; Vogelstein et al 2013 (*Vog-2013*) → ≈ 4 x 10^{-97}. Even by adjusting for gene coding sizes of 1000-2000 bp, the values for the most credited models are (per gene) *Hoc* 2011 → 10^{-50} and *Vog*-2013 → 10^{-60} and by accepting very large families for each of the hallmarks, per-gene families: *Hoc* 2011 → 10^{-30} and *Vog*-2013 → 10^{-36} [8, 61]. The values are so low, that they would difficultly accommodate for carcinogenesis in *H. sapiens* (or other species).

Question 3. Darwinian vs Adaptive (and additional) Models. This is why the majority of cancer models today invoke additional means for generating diversity [6, 10]. One of the first models that increased variation in the *L-D model* was developed by John Cairns, as the *Adaptive Mutation Model* (*Adapt-Mut Model*) [62-63]. Its experimental definition was based upon a deletion of the *Lac Operon* and a *frameshift* mutation in the *Lac-I/Lac-Z* fusion gene carried in a *F'-lac* plasmid: if *E. coli* is then fed lactose as the only source of energy, the *frameshift* mutation is reverted either directly or by amplification of the mutated gene at higher frequency (*Adapive Mutations*) [62-63]. Similar models have been studied by J. Roth [64-66], SM Rosenberg [67-69] and PL Foster [70-72]. Unfortunately, the issue is still controversial [68, 73-74], since at least two positions are held (rather strongly): 1. Adaptation Selection Model (*Adapt-Sel Model*): the groups of John Roth, D. Anderson and others affirms that the strong mutation increase under selection could be simply explained by selective forces [65, 74], a position similar to that of W. Bodmer against L. Loeb's *mutator phenotype* (*Mut-Phen Model*, see next section); 2. Adaptation Hypermutation Model (*Adapt-Hyper-Mut*): the data accumulated initially by John Cairns (also with P. Foster) and later by the groups of PL Foster, SM Rosenberg and others support a vision in which a "*hypermutator*" phenotype is activated in stationary cells under starvation (for example, for lactose as energy source, in the pioneering Cairns-Foster experiment) and even transiently. Therefore, two mechanisms were proposed: 2a) An original model proposed by Foster-Cairns suggested that mutagenesis was directed to sites that improve growth (so that the model was sometimes referred to as *Directed Mutation– "Direct-Mut Model*"). That this is not so was eventually corrected also by Foster and Cairns and other groups: bacteria under stationary and starved

growth conditions (for example, for Lactose), do not mutate only *Lac-Z*, but also other genes present in the *F'-lac* plasmid, for example mutants for *Tetracycline resistance -* (to→ *Tet-A+*). 2b) In recent years, a mutator model has been perfected by PL Foster and SM Rosenberg (*Adapt-Hyper-Mut*): cells carrying such a phenotype typically i) display presence of unselected mutations and ii) appear to be present as a smaller fraction of the bacterial population (i.e., 1/1000). This is certainly not trivial: in a typical experiment with 10^8 plated cells, since the number of scored revertants is very high (100), mutation frequency appears to skyrocket to 10^{-3} (! : i.e. 10^2 in a population of 10^5) [75]. In fact, this could be the highest frequency of mutation ever recorded, even higher than that induced by any chemical, mutagen etc. ! [76] Both lab.s have also documented the potential role of recombination system(s)(*RecA, Uvr ABC*) in these adaptive mutations, with further potentially important implications for eukaryotic and cancer cell [69, 72].
Some of these data were also extended to eukaryotic cells by T. Tlsty [77] and others: in some of these models, gene amplification could be considered an "adaptive mutation" of tumour cells [78-80]. However, "*what would these tumor cells adapt to*" still remains an open question.

Question 4. Mutations vs Selection (or something in-between). In Darwinian models, we distinguish between casual (i.e., random)-mutations (MUT) and extrinsic selection (SEL): how can we distinguish in cancer onset/progression between the two and especially what is the "*cancer-engine*" selecting for ? The long debated question of prevalence of *Mutations vs Selection* appears to be still unsolved, or at least the proposed solutions are often at the opposite scale-extremes. Just two examples: W. Bodmer has often emphasized the importance of selective forces in cancer onset and progression, see for example his paper with Tomlinson: *"Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog"* [81] or his strong criticism of the *Mutator Phenotype* by L. Loeb (*Mut-Phen Model*) [82-83], therefore minimizing the importance of increased genetic instability (either exogenously or endogenously generated) [84]. Questions remain about i. what exactly such selective forces are for inducing cancer and for its progression [10] and ii. Why not all develop cancer ? : since we should be all subjected to the same/similar selective forces [6] . At the opposite end, the two recent papers by Tomasetti and Vogelstein in Science [85-86] (*T-V Model*) emphasize the stem cell targets and clearly show that larger stem cell compartments -in terms of total cell divisions and therefore mutation rates- correspond to increased cancer risks (for 2/3 of analyzed tumor types). It should be underlined that the great controversy generated by these papers and the general perception/understanding of this *T-V Model* [87] risk to create the typical "*storm in a tea-cup*" ("*espresso cup*" in this case). It should be not surprising that stem cell compartments with much higher cell divisions and therefore mutations generate tumors with higher frequencies. Real questions remain on why different life-stiles dramatically affect cancer rates [27] (and therefore also the underlying dividing stem cell compartments –or some of them), as innumerable epidemiological studies -on migrant populations for example- have shown [27]. Therefore, the two positions, Bodmer's and T-V, just reflect different lenses or angles of looking at similar questions, another "*five-blind-men and the elephant*" story.

Question 5. Extreme Longevities. Beside the discussed *PP*, which states that carcinogenesis remains approximately at similar values also in large-bodied and long-lived animals, the existence of extreme longevity in a few species is posing new questions. For example, the naked mole rat (*NMR*) with its 20 years and the blind mole rat (*BMR*) with 32 years record age show at least 20X and 32X longevity increases, compared to *Rattus rattus* and other *Rattus* species. Such species with extreme longevity pose a logical dilemma in terms of the underlying biological mechanisms: here longevity appears to be dissociated from cancer-risk increases (i.e., accrual of mutations). In fact, longevity is here associated with increased resistance (not incidence) to cancer. Data obtained by Seluanov and Gorbunova document cellular mechanism(s) associated with cellular senescence, telomere/telomerase regulation, early contact inhibition (ECI) and presence of much longer hyaloruran polymers –HMM-HA- [88-90]. The problem therefore also intersects the question of aging, which appears in these models inversely proportional to cancer incidence (*NMR Model*). Aging, however, was shown to be essentially irrelevant for carcinogenesis in the original paper by Richard Peto and elsewhere (quoted for *Peto's Paradox, PP* [24], see also [26]). Here, carcinogenesis administered through skin application of benzopyrene (BP) at 10, 25, 40 or 55 weeks of age caused essentially the same number of skin carcinomas, irrespective of age of the rats. In fact, additional carcinogenesis experiments using phorbol esters (tumor promoters) or nitrosamines (1ary carcinogens) indicated that carcinogenesis may be less efficient (i.e., lower incidence) in older age [91] [92]. The *NMR* and *BMR* models therefore pose questions to our current understanding of carcinogenesis and suggests that strong selective forces (i.e., the peculiar exclusively underground habitat of this species, rather uniform temperatures, humidity etc) have molded its genetic constitution and cancer-resistance [88, 93]. A similar parallelism has been sketched by Robert Gatenby considering the evolution of eye-less cavefish species in several regions of Mexico and Central America [94]. It is clear that such mutations have been strongly selected by the habitat in different species and with different although obviously convergent pathways. These two examples underline, although with different angles (cave-fishes in more indirect way), the importance of selective forces "molding" the genetic traits in cancer-onset and progression [94-95]. They also stress our relative ignorance in understanding the forces that operate in cancer cells for molding the so called "cancer genomes" [88, 93].

III. **Epistemology.** Cancer modeling also belongs to epistemology and an epistemological-view of cancer modeling today could be proposed, especially taking into account the falsification principle by C. Popper and Historical-Cultural valuations by Th. Kuhn. The previously discussed Mukherjee text together with an excellent more recent overview-documentary by Ken Burns on the same subject suggest that such a reflection may be appropriate. Falsification should be used in the spirit of modern scientific thought born around 1543, subsequently developed by Galileus and typically characterized by a sequel of steps: question→hypothesis→predictions→experiments→analysis (QHPEA) [96]. Falsification or refutability principle by Popper should be also applied to cancer modeling according to the same or similar schemes. However, some of today's proposed models are epistemologically weak, in the sense that it would be

difficult to apply the falsification principle. *HoC* for example shares several weaknesses with other models, in the sense that it is not clear how many steps are necessary/sufficient for malignant transformation. In the classical or modified *HoC*, should a cell undergo each of the 6 or 10 mutational steps in order to become fully malignant and metastatic ? Although very interesting work had been initially performed by Hahn and Weinberg in the past (using a limited number of mutational steps) [97-99], no one to my knowledge has further mutated a "completely normal" human or mouse cell in the 10 different pathways of the hallmarks (U. Rovigatti, manuscript in preparation). All this notwithstanding, refutability could be adopted according to contradictions or obvious paradox of the model itself (see section II.1 Peto's paradox) and also according to the QHPEA logical scheme applied to the model implications [96] [22]. As discussed in I (Previous models as metaphor), an aerial view of cancer research today –as it has been done for example by Mukherjee and Burns- clearly indicates a strong association between cancer modeling and suggested/implemented strategic therapeutic interventions. Therefore the QHPEA analysis could be also applied to the therapeutic application of the model: for *HoC* this is unquestionably the so called targeted-gene-therapies (*TGTs*) . *TGTs* appear today insufficient, not-resolving and *ephemeral*, in the sense that they typically induce shorter remission times [6] [10]. According to this logical scheme – which explains cancer as consequence of a limited number of genetic mutations - they should be instead –in order to demonstrate correctness of the model-: sufficient, completely resolving and durative. The fact that *TGTs* do not cure cancer patients, by completely blocking or stopping the disease, falsifies in logical terms the initial model: it suggest that it should be modified or corrected (U. Rovigatti, 2016; U. Rovigatti, manuscript in preparation).

One second and interesting angle-view for considering cancer modeling is through the lenses of the American epistemologist Thomas Kuhn and his well known *"The Structure of the Scientific Revolutions" TSSR* [44]. Cancer modeling in general is well suited for such an analysis, also in view of the strong connections with the cultural/social beliefs of the historical period (see part I, "previous modeling as metaphor"). Accordingly, today's prevailing model (*HoC*) appears to be a typical progress of "normal science" or "paradigmatic phase", with several "anomalies" already appearing as it was documented in II (*5 simple questions still unanswered by HoC or cancer research in general*). According to *TSSR*, attempts to resolve anomalies are typically made with scientific instruments already present in the same normal-science or theory, but they accumulate to the point of reaching a "paradigm shift", therefore leading to the model/theory reappraisal and creation of new paradigm [44]. The present insufficiency/failure of *TGT's* and of the underlying theoretical models, suggest that cancer modeling is presently undergoing a phase of anomalies-accumulation and therefore leading to future paradigm-shifts.

IV. **10 different models: Static vs Dynamic models.** It is apparent that *HoC* has had an essential role for almost 20 years in aggregating and condensing several different areas of molecular biology of cancers cells [7, 8] – so called molecular oncology- but that today different directions or explicatory mechanisms are emerging, so that an aerial view of cancer research today should not count just one *(HoC)* but rather 10 different cancer models. This was previously suggested

by me in two papers [6] [10] and is discussed in more depth in a manuscript in preparation. Accordingly, these are the broadly defined models: 1. Genetic Models (*CAN-GEN such as HoC*); 2. Epi-Genetic Models (*CAN-EPI*); 3. Chromosomal Models (*CAN-CHROM*); 4. Genetic Genomic Heterogeneity (*GGH)*; 5. Evolutionary Ecological (*EE*); 6. Epidemiological; 7. Cancer Stem Cell (*CSC*); 8. Immunological (*IM*); 9. Micro-Environment (*ME*); 10. Combinatorial (*CO*) [6].

Instead of addressing each model separately or establishing priorities of one vs another, I contend that cancer research today may benefit by considering all available ten models with a sort of "*quantistic*" approach. In other words, this may help in searching the "*engine of cancer*", with alternative strategies and in order to finally find convincing solutions, also capable of explaining the questions discussed before.

In this sense, it may be important to distinguish between *Static* vs *Dynamic* models. *Static models* are here defined as models which give a definition of the essential factors present in cancer cells. They provide an association between the malignant phenotype and a certain number of cellular parameters. *Dynamic models* instead consider carcinogenesis, especially human, as an ongoing and developing process, for which it is difficult to define essential and required genetic events or phenotypes. The emphasis is therefore more on the underlying mechanisms generating the cancer-phenotype and genetic-heterogeity. Finally, *Bivalent models* consider both static and dynamic elements in different phases of the disease. An instructive example to distinguish between *Static vs Dynamic* Models is to consider how genetic/genomic instability is considered by these two different explicatory mechanisms, for example in *HoC* and *GGH/EE*. In *HoC* [8], *Genomic instability and mutations* was not even considered and listed as one of the *Hallmarks* in the "first edition" of 2000, and added as one of the two *Enabling characteristics* in the 2011 version [7] [8]. Furthermore and most of all, it appears to be just one of the 10 elements (8 *hallmarks* and 2 *characteristics*) characterizing a cancer cell. In *GGH/EE* instead, *Genomic instability and mutations* appear to be the essential engine of tumor development and progression (*GGH*) [61, 100] or the most important pattern or behavior for defining the tumor cells (*EE*) [101-102]. The epitome of a *Static* model is the one by Tomasetti-Vogelstein (*T-V Model*): there should be a certain number of mutations in Stem Cells (*SC*s) and these are shown to be approximately proportional to the number of divisions for 2/3 staminal cell compartments [85-86]. Once such threshold is reached and passed, cancer is elicited: it could be by mutagens, ionizing radiations or just by random replication problems: their data would suggest a prevalence of replication errors [85-86]. In *Dynamic models* instead, the mechanism(s) for accumulating mutations is an essential component of cancer origin: it is the cancer problem itself. The consequences for therapeutic approaches are also relevant: in *T-V*, since it does not matter how mutations were accrued, the relative therapeutic approach will be entirely based upon the tumor *Landscapes* and therefore on *TGTs* or similar therapies, with only little/some provision for tumor evolution pathways [6]. In *Dynamic* models, since this mechanism of variation/heterogeneity-generation is active in tumor cells and considered their essential problem, therapeutic intervention(s) will have to be defined and are still being investigated. Factors affecting *evo-eco* of tumors cells and tumor classification schemes accordingly are privileged [102]. This is why

Dynamic models privilege today an observational/predictive and research-oriented approach, in order to identify appropriate biological targets in the future [6, 101-102].

Fig. 2. Static vs Dynamic vs Bivalent Models from Today's Cancer Research

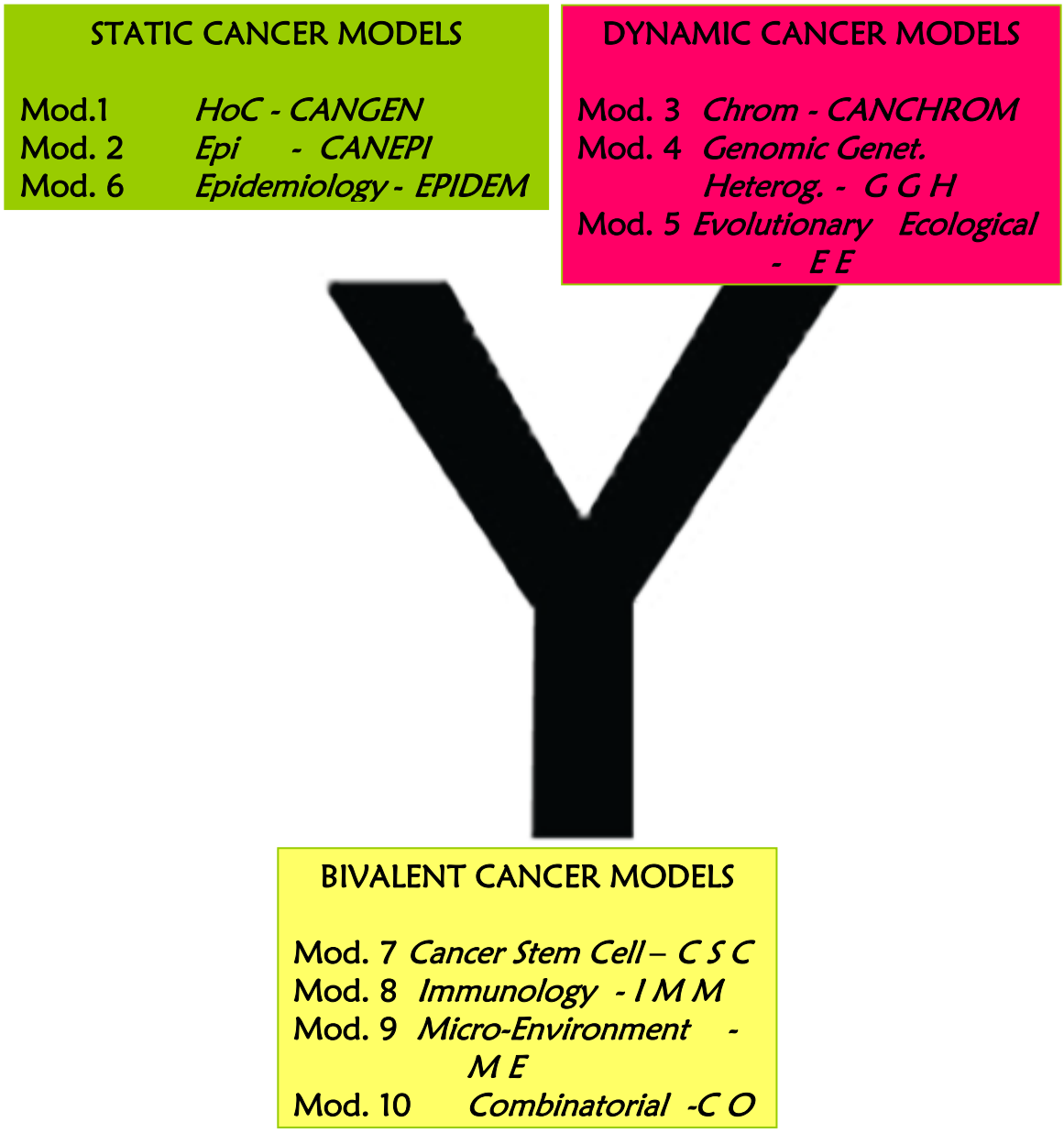


Figure 2. Models are numbered according to (Rovigatti 2015) Rovigatti, U. (2015). "Cancer modelling in the NGS era - Part I: Emerging technology and initial modelling." Critical Reviews in Oncology/Hematology **96**(2): 274-307. Acronyms are described in the text. Essential references are in Table I of Rovigatti 2015.

V. Discussion, future directions and conclusions

This brief overview of present problems in today's cancer modeling suggests that there are clearly areas where we should see improvements in the near future. More than suggesting solutions, this review wanted to underline the existing problems. The described 5 easy problems are often ignored or dismissed: however they stand out in today's molecular understanding of cancer as logical paradoxes. First of all the one named from Sir Richard Peto (PP): although several molecular solutions have been suggested especially in the last few years, none is complete, general in terms of Darwinian evolution and most of all capable of explaining the very large gap of PP. The described low incidence of new mutations is often ignored or even opposed despite the logical evidence: that cancer cells increase such very low background level is unquestionable despite today's model invoking normal physiology or bad luck [83-86] . Another aspect often ignored is the seminal observation by John Cairns that not just Darwinian (i.e. random) but also adaptive mutations are generated [62-63]. Controversies in this area may explain delayed acceptance, but concepts such as hyper-mutating subpopulations are extremely interesting and deserve further analysis. Diatribes over nature vs nurture are quite similar, but typically addressed by extreme podiums and with accents more typical of politics than science and logics. Finally, an extremely interesting and presently fecund area has been initiated by studying very high longevity in a few species. The focus here has been on the Naked and Blind Mole Rats (*NMR/BMR*) for potential comparisons with other more familial rodents (*rattus rattus* and *mus muris*). Interestingly, such comparisons at the genetic and genomic levels, are now appearing in the literature [88, 93].

The existence of such 5 simple unexplained anomalies or paradoxes could be reconciled by an epistemological view of today's cancer modeling. An eagle eye or aerial view suggests that the present models cannot substantially improve our understanding or the therapeutic intervention success. There are several potential explanations for this and I also previously suggested that mechanism upstream (called *UPCAN*) to the present layer and particularly the dominant ones (called *GENOME STRIPERS*) are still poorly studied today in comparison to a merely descriptive Landscape (called *CANGEN*) of derangements [10]. From this, the need today for expanding our analysis and interpretation of cancer not just with one paradigm but with several potential models (I have listed 10). The distinction between Static vs Dynamic models is logically due also in view of the previous pitfalls, as Static models tend to just depict the cancer cell Landscape while Dynamic ones focus on the mechanism(s) behind determining such tumor cell heterogeneity. The deduction of 9 additional models from cancer research work actively discussed in today's literature follows the need for modeling improvement and especially for a better definition of the "cancer engine": what fuels cancer progression and tumor heterogeneity. With the same token, the distinction between Static vs. Dynamic models simply characterizes their scope and does not imply a quality difference or suggest that one type will provide the final answer to cancer. In this final search for a solution, bivalent models (containing both static and dynamic aspects) are also potentially interesting solutions.

The logic behind this overview is therefore that of expanding the realm of cancer modeling, in order to obtain better/final solutions in a time of uncertainty about cancer interpretation and therapeutic modalities. The only certainty we have today is that we must improve our cancer interpretation in order to achieve a better management of aggressive malignancies, which are still untreatable.

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